#1127 THE INSTIUTE OF PAPER CHEMISTRY
(A Study of Coloring Matter in Woods)
Project Reports

PROJECT REPORT FORM

PROJECT NO. 1127
COOPERATOR Institute
REPORT NO. 10
DATE March 7, 1959
NOTE BOOK 1426
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SIGNED M. A. Buchanan
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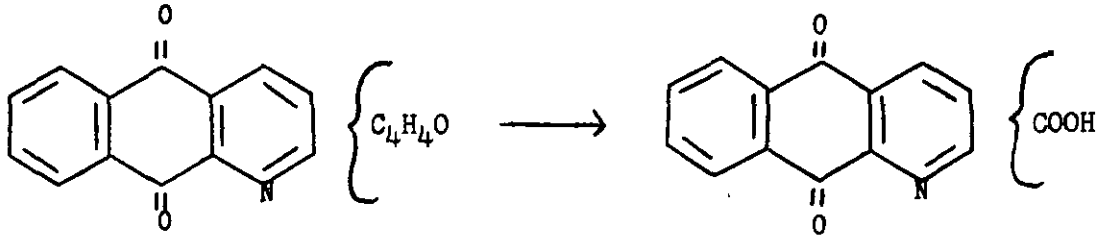
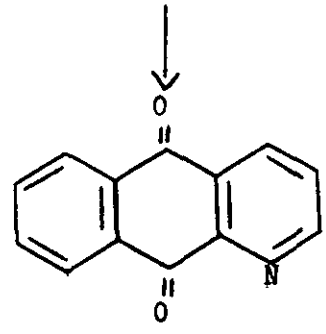
OXIDATION OF THE YELLOW PIGMENT FROM YELLOW POPLAR

Project Reports No. 5, 6, 7, and 8 described the isolation and study of a yellow pigment with a melting point of 282° from yellow poplar heartwood. Analysis had indicated the pigment has the composition $C_{17}H_9O_3N$. Previous attempts to degrade this material to known compounds or to form known derivatives were not successful. This report describes oxidation of the yellow pigment to an acid having the composition $C_{14}H_7O_4N$ which is decarboxylated at the melting point to form benzo[g]quinoline-5,10-dione (1-azaanthraquinone). Thus a partial structure of the pigment can be formulated, and the degradation pictured as:



$C_{17}H_9O_3N$
Yellow Pigment

$C_{14}H_7O_4N$
Oxidation Product



Benzo[g]quinoline-5,10-dione

The location of the carboxyl group in the acid obtained by oxidation and the location and nature of the fourth ring in the yellow pigment are still uncertain.

OXIDATION WITH CHROMIC ACID

In Project Report No. 7, Mr. Dickey described the oxidation of the yellow pigment with potassium permanganate in a hot 10% sulfuric acid solution. A small amount of crystalline material was obtained which melted at 330-2°C. with decomposition and formation of a crystalline sublimate. Because of the very low yield, the product was not investigated further. Attempted oxidation with chromic acid in acetic acid solution failed to give any indication of oxidation.

Further attempts to degrade the yellow pigment have resulted in conditions which give a high yield of the material isolated first by Mr. Dickey. The yellow pigment can be oxidized with chromic acid in sulfuric acid solution, but the reaction conditions appear to be critical. A rather high concentration of sulfuric acid is necessary to prevent the formation of a red-colored insoluble material which is resistant to oxidation. On the other hand, oxidation does not stop at the desired point when the concentrated sulfuric acid solutions are heated. The following conditions give a yield of about 80% of the desired product:

> The yellow pigment (0.1 g.) is dissolved in 8 ml. of 1:1 (v./v.) concentrated sulfuric acid-water by warming, and the solution diluted with 3 ml. of water. After cooling to room temperature a solution of 0.2 g. of chromic oxide dissolved in 1 ml. of water and diluted with 4 ml. 1:1 sulfuric acid-water is added gradually over a period of about an hour. After standing at room temperature for 15-16 hours, the solution is diluted with an additional 40 ml. of water, and the mixture is heated in a large test tube in a steam bath for 1 hour. After cooling to room temperature, the reaction mixture is filtered to give a light colored product which is dissolved in dilute ammonia, and is reprecipitated by acidification with concentrated hydrochloric acid. The crude products have a melting point of about 330°, and on melting always form a sublimate of yellow colored needles.

CHARACTERIZATION OF THE OXIDATION PRODUCT

The oxidation product had poor solubility in most of the neutral solvents tested. It was soluble in hot formic, acetic, or nitric acids, but satisfactory recrystallizations from these were not obtained. The product was purified by dissolving in approximately 2% ammonium hydroxide, filtering, heating the solution, and then neutralizing with hydrochloric acid. After several such treatments, the melting point was 333°C. on rather rapid heating. The purified material was analyzed by Huffman Microanalytical Laboratories:

	Found	Calc. for $C_{14}H_7O_4N$
Carbon	66.33	66.41
Hydrogen	2.83	2.79
Nitrogen	5.60	5.53
Residue	0.35	

These results along with the solubility in ammonium hydroxide indicated that the oxidation product was a monocarboxylic acid which had been formed by opening a ring followed by the loss of three carbon atoms.

When the oxidation product was heated to the melting point, gas was evolved and yellow needles formed in the cool part of the tube. The needles had a melting point of about 270°, and were believed to be formed by decarboxylation of the acid. Small amounts of the needles were obtained by mixing 10 mg. of the oxidation product with 20 mg. of precipitated calcium carbonate and heating the mixture to 235°C. The needles formed in the upper part of the tube, but the yield was low.

The melting point suggested that the needles might be benzo[g]quinoline-5,10-dione, and a small amount of this material was synthesized by the method of Clemo and Driver (1). Mixed melting point and infrared spectra indicated that the needles and the synthetic product were identical. The needles, the synthetic product, and a mixture of the two all melted at 275-7°C. Clemo and Driver reported a melting point of 276° for their product.

Both the oxidation product and the sublimed needles dissolved in concentrated sulfuric acid and formed yellow-colored solutions in contrast to the rose-red color of the original pigment in sulfuric acid. The oxidation product was soluble in 1% sodium hydroxide, and the solution gave an immediate blue to purple color on addition of sodium hydrosulfite. On standing the color changed to red, to yellow, to colorless, but was restored by additional hydrosulfite. The sublimed needles were insoluble in sodium hydroxide, but on addition of sodium hydrosulfite gave coloration similar to the above.

REFERENCE

1. Clem. G. R., and Driver, G. W., J. Chem. Soc. 1945:829-33.

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ISOLATION OF A SECOND YELLOW PIGMENT FROM YELLOW POPLAR HEARTWOOD

A small amount of a second yellow crystalline substance has been isolated. This substance can be crystallized from ethanol, and has a melting point of 236°. It dissolves in concentrated sulfuric acid to form a strong red color. It is quite similar to the first yellow substance as regards: chromatographic behavior, basic properties, and absorption spectra (to be reported later). It differs from the first substance in that it contains four methoxyl groups. Equivalent weight data suggest that it is derived from the first substance by the replacement of two hydrogens with methoxyl groups and the replacement of two oxygens by methoxyls. Larger amounts of this second material need to be isolated before it can be characterized further.

EXPERIMENTAL

The final mother liquor from crystallization of the first yellow substance from 4800 grams of yellow poplar wood (Project Report No. 8) was evaporated. The residue which amounted to 1.45 grams was washed with 15 ml. of 95% ethanol. The ethanol-insoluble material (0.80 gram) was dissolved in 1.5 ml. of hot chloroform, but nothing crystallized on cooling. The chloroform was evaporated, and the residue was dissolved in about 40 ml. of hot 95% ethanol, and the hot solution was filtered. On cooling, 0.300 gram of orange needles separated. These darkened at 220° and decomposed at about 230°. The needles were recrystallized from 20 to 25 ml. of ethanol. The resulting crystals amounted to 0.265 gram and melted at 233-6° with decomposition. They were insoluble in water, but were readily soluble in dilute hydrochloric acid to form a red colored solution. After two additional crystallizations from ethanol, the crystals amounted to 0.193 gram and melted at 235-6° with decomposition. A sample was dried in an Abderhalden and was submitted for analysis. The yield of the crude crystals was 0.006% based on the original sawdust. Since there were probably losses, it seems likely that the wood contained about 0.01% of this substance.

Analytical data for this substance are given in Table I. Analysis for nitrogen by a Kjeldahl procedure failed to indicate the presence of nitrogen, but a qualitative test for nitrogen did show the presence of nitrogen. The equivalent weight was determined by a nonaqueous titration.

TABLE I

ANALYTICAL DATA FOR YELLOW SUBSTANCE M. P. 236°

Carbon, %	68.54	68.01
Hydrogen, %	4.90	4.96
Methoxyl, %	33.54	33.50
Equivalent weight	359	360

The second yellow substance was chromatographed on multibuffered paper in the manner described in Project Report No. 8. There was a strong yellow spot with yellow fluoresence in the pH 5.0 zone. There was also some fluoresence at the starting point and in the pH 6.4 and 6.2 zones.

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THE YELLOW PIGMENT FROM YELLOW POPLAR WOOD WITH M. P. 282°

Project Reports No. 5, 6, and 7 described the isolation and study of a yellow crystalline substance from the heartwood of yellow poplar. This report describes the isolation of additional quanities of the material and some experiments directed toward the characterization of the substance.

Analytical data indicate that the yellow substance has the composition $C_{17}H_9O_3N$. The low ratio of hydrogen to carbon suggest that the substance is built up of condensed rings. Formation of a mono oxime indicates that one of the oxygen atoms can exist in the grouping C=O, but this may be tautomeric with a hydroxyl form. The nature of the other two oxygen atoms is less certain, but reduction tests suggest that they are present as an anthraquinone grouping.

EXPERIMENTAL

ISOLATION OF THE YELLOW CRYSTALLINE MATERIALS

Project Report No. 6 described the isolation of a yellow crystalline material which melted at 280°. Several additional isolations were carried out in order to obtain more of this material for study. Different methods of isolation were tried, and the following was judged to be the best of the procedures tested.

Sawdust prepared from yellow poplar heartwood was extracted with benzene-95% ethanol 10:1 in a Soxhlet apparatus for 6 hours. A second charge of sawdust was then extracted with the same solvent for 6 hours. The extract from the two charges of sawdust was concentrated under reduced pressure to about half of its original volume. This removed the ethanol and caused the precipitation of some dark colored material which was separated by filtering. The resulting benzene solution (about 1.5 l.) was extracted 5 times with 100 ml. portions of 1% hydrochloric acid. The combined acid extracts were neutralized by the cautious addition of solid sodium bicarbonate, and the liberated bases were extracted with chloroform. The combined chloroform solutions were evaporated under reduced pressure to give a crystalline residue of impure material, which is referred to as the crude basic fraction. This fraction was recrystallized from chloroform (4 ml. per g.) to give reasonably pure yellow crystals with a melting point between 270 and 280°. Only 10 to 20% of the crude basic fraction was recovered as crystalline material on recrystallization. Small additional amounts of the yellow crystals could be obtained by chromatographic purification of the mother liquor solids, but the mother liquor contained mainly other materials.

The precipitate obtained on concentrating the original extract contained some yellow materials which could be extracted with dilute hydrochloric acid or with chloroform, but only traces of the desired crystalline material appeared to be present. This fraction was usually discarded. Some yellow colored impurities could be separated by washing the concentrated benzene solution with water, but this step did not seem to be worthwhile. Both 10% and 0.5% hydrochloric acid were tested for the extraction of the benzene solution. The stronger acid seemed to promote formation of some red colored, insoluble material, and extraction with the weaker acid seemed to be incomplete. Consequently, the use of 1% acid was preferred. In the early experiments, the neutralized aqueous layer was extracted first with benzene and then with chloroform. Benzene extracted most of the desired yellow material but not all of it. Since extraction with chloroform was needed to remove all of the material, the benzene extraction seemed needless.

Different lots of yellow poplar heartwood were extracted. One lot consisted of well seasoned lumber obtained from Standard Manufacturing Company. Seven extractions on this wood gave an average yield (based on the air dry wood) of 0.3% crude basic fraction and 0.06% recrystallized material. In another case, unseasoned yellow poplar logs were obtained in June, 1956 from West Virginia Pulp and Paper Company. Sawdust prepared from the heartwood was divided into two parts. One half of the moist sawdust was extracted directly, and the other half was air dried before extraction. Average yields (oven dry basis) from five extractions of each sample were 0.6% crude basic fraction and 0.08% recrystallized material from the undried sawdust, and 0.6% crude basic fraction and 0.07% recrystallized material from the air dried sawdust. Thus, there was a slight advantage in extracting the undried sawdust.

The mother liquors from the crystallization of the crude basic fractions contained 80% or more of the solids from these fractions, and were strongly colored. In an effort to recover more of the yellow crystalline material, the mother liquor solids were chromatographed on alumina columns using chloroform as the developer. The effluent collected ahead of the main yellow band was separated as a first fraction. This consisted chiefly of a colorless effluent, but near the end of the collection the effluent was yellow to brown, in color. Evaporation gave a brownish colored glass-like residue which amounted to 40 to 80% of the mother liquor solids. A second fraction amounting to 20 to 65% of the mother liquor solids was obtained by evaporating the solvent from elution of the main yellow band. This fraction was crystallized from chloroform and yielded additional yellow crystals with a melting point of about 280°. However only 3 to 8% of the fraction was obtained as crystalline material. A third fraction was collected after the main yellow band. The first part of this fraction consisted of a green colored solution, but much of the material consisted of a brown colored solution. The third fraction was small, amounting to only 1 to 3% of the mother liquor solids.

The alumina purification of the mother liquor solids was applied to six of the extracts from the seasoned lumber. The mother liquors from crystallization of the second fraction obtained in one separation were combined with the mother liquor from the crystallization of the crude basic fraction from the next extract, and the alumina separation was repeated. Thus there was a tendency for the second fraction to build up as the separations were continued. Additional yellow crystals melting at about 280° were isolated in an amount corresponding to 0.006% of the wood in these treatments. A second yellow pigment has been isolated from the final mother liquor from these treatments. This is to be described in a later report.

PURIFICATION

The crystals obtained from the first crystallisations melted somewhat below 280°. Additional recrystallizations from chloroform raised the melting point to 280°. For recrystallization of the relatively pure material, 35 to 60 ml. of chloroform were used per gram.

In order to obtain high purity material for analysis, the recrystallized material was chromatographed on alumina using chloroform as the developer. The effluent from the main yellow band was evaporated and the residue recrystallized again.

In one case, 85 mg. of crystals from the alumina purification were heated on the steam bath with 15 ml. of 95% ethanol. Very little material dissolved, and benzene was added gradually. Most of the material dissolved after the addition of 2 ml. of benzene, and the hot solution was filtered. The material separated in the form of small balls on cooling. The mixture was reheated, and the material redissolved after the addition of 1 ml. of benzene. The solution was concentrated to 6 to 7 ml. on the steam bath, and then was cooled slowly. Needle-like crystals separated which melted at 281°. These crystals were greenish-yellow in color, and appeared to be somewhat lighter in color than the crystals from chloroform.

In another case, crystalline material which had been crystallized three times from chloroform was purified on an alumina column and then was recrystallized again from chloroform. These crystals melted at 282° and were used for analysis.

OXIME

A 0.257 gram sample of yellow crystals with a melting point of 280-1° were heated on a steam bath with 5 ml. of pyridine and 0.267 gram of hydroxylamine hydrochloride. The material did not dissolve, and after 15 minutes, 5 ml. additional pyridine were added. After 30 minutes, the material was still undissolved, and the mixture was heated on a hot plate under reflux. The material dissolved after refluxing for 10 minutes. The mixture was refluxed for a total time of 30 minutes, and then was stirred into 100 ml. of 2N acetic acid. The yellow precipitate was filtered, washed with water, and dried. The yield was 0.267 gram. The product was only slightly soluble in hot chloroform, ethanol, n-propanol, or n-butanol, but was somewhat more soluble in hot benzene and chloroform. It was crystallized twice from n-butanol using about 70 ml. of solvent per 0.1 gram of material. The final product had a melting point of 271°.

ANALYTICAL DATA

Analytical data for the yellow substance which were given in Project Report No. 6 did not agree with calculated values for any relatively simple substance. Analysis of subsequent samples gave variable results. The earlier samples were probably not entirely pure, but combustion of this material was very difficult and it seems likely that the analytical procedures used were not entirely satisfactory for analysis of this compound. Finally, the material with a melting point of 282° and the oxime were submitted to Huffman Microanalytical Laboratories, P.O. Box 125, Wheatridge, Colorado. The Huffman data for the first yellow substance for carbon, hydrogen, oxygen, and nitrogen total very close to 100 per cent, and they agree very well with the values calculated for the formula $C_{17}H_9O_3N$ (Table I). Carbon and hydrogen data obtained at the Institute for the sample crystallized from ethanol also agree with the above values.

The data for the oxime approximate the values calculated for a mono-oxime prepared from a compound with the formula $C_{17}H_9O_3N$. The residue from the carbon and hydrogen determination suggest that combustion was not complete, and that this may have been responsible for the low carbon value.

TABLE I

ANALYTICAL DATA

Yellow Substance m.p. 281-2°

	Crystallized from ethanol		Crystallized from chloroform (1)		Calc. for $C_{17}H_9O_3N$
Carbon, %	73.92	74.21	74.17	73.94	74.18
Hydrogen, %	3.31	3.39	3.28	3.36	3.30
Oxygen, %				17.52	17.44
Nitrogen, %			5.07	5.08	5.09
Mol. Wt. (2)				270	275.2

Oxime (1)

		Calc. for $C_{17}H_{10}O_3N_2$
Carbon, %	69.68	70.34
Hydrogen, %	3.34	3.47
Nitrogen, %	9.45	9.65
Residue from carbon and hydrogen, %	0.5	

(1) Huffman data
(2) By an ebullioscopic method using chloroform as the solvent.

BEHAVIOR WITH ACIDS AND ALKALI

The yellow substance is soluble in dilute mineral acids and in aqueous acetic acid, but the rate of solution is rather low at room temperature. The substance dissolves readily in concentrated sulfuric acid to form a rose color which is rather stable on standing.

A few crystals of the yellow substance were mixed with 10 ml. of 1% sodium hydroxide. There was no apparent change at room temperature within 10 minutes. The mixture was heated on a steam bath for 1 hour. This developed a red color, but the reaction appeared incomplete. The mixture was refluxed for 2 hours. The final reaction mixture consisted of a red colored insoluble material. This product was not soluble in chloroform. After neutralization, the product was still insoluble in chloroform but was slightly soluble in ethanol and in dioxane.

HYDROCHLORIDE AND BROMIDE

A hydrochloride was prepared by dissolving 79 mg. of yellow crystals in 10 ml. of 10% hydrochloric acid on the steam bath. Absolute ethanol (40 ml.) was added, and the hot solution filtered. On cooling in the freezing compartment of the refrigerator, the filtrate deposited red needle-like crystals. These were recrystallized by heating with 25 ml. of 95% ethanol and cooling the filtered solution in the freezing compartment. The final brick red crystals amounted to 44 mg. and had a melting point of about 268°.

A dilute solution of bromine in chloroform was added dropwise to a small amount of the yellow crystals dissolved in chloroform. A dark red precipitate formed which was insoluble in water, but was soluble in dilute hydrochloric acid forming a yellow colored solution. The product was soluble in hot absolute ethanol, but was only very slightly soluble in hot chloroform.

Neither the hydrochloride nor the bromine reaction product were investigated further.

CHROMATOGRAPHY ON MULTIBUFFERED PAPER

A chloroform solution containing about 2 mg. of the yellow substance per ml. was spotted on paper impregnated with buffers in the range of pH 6.4 to 4.2, which were prepared by the procedure described by Schmall, Wollish, and Shafer (1). The paper was developed with chloroform. The material stopped in the pH 5.0 zone, and was a dark greyish color without fluoresence. A known solution of codeine had been applied to the paper, but the codeine spot was not visible in ordinary light or in ultraviolet light. The location of the codeine was not detected on this chromatogram, but under similar conditions codeine was said to stop at the pH 4.8 zone (1). Thus it seems likely that the basic strength of the yellow substance is in the range of that of codeine.

TESTS FOR METHYLENEDIOXY GROUP

About 10 mg. of yellow crystals were dissolved in 8.5 ml. of water and 1 ml. of 10% sulfuric acid, and 4 ml. of concentrated sulfuric were added. The mixture was heated and 3 ml. of distillate were collected in a test tube cooled with ice water. A test (2) for formaldehyde was made by adding 0.2 ml. of 1% chromotropic acid solution to 1 ml. of distillate, cooling in ice water, and adding 5 ml. of concentrated sulfuric acid. No color developed, indicating a negative test. Under the same conditions, the distillate from piperonal gave a strong reddish color immediately. The above reaction mixture from the yellow crystals were heated again, and a second 3-ml. portion of distillate collected. This second distillate gave a moderate bluish color in the formaldehyde test. Finally, the mixture was heated again and an additional 2 ml. of distillate collected. This gave a very strong bluish color in the test.

In another test, 10 mg. of the yellow crystals were dissolved in 7.5 ml. of water and 1 ml. of 10% hydrochloric acid, and 3.5 ml. of concentrated hydrochloric acid were added. This solution was also distilled, three 3-ml. portions of distillate being collected. None of these solutions gave a test with chromotropic acid and sulfuric acid.

The yellow crystals also failed to give the Labat test (3) for the methylenedioxy group. In this test, a small amount of the crystals were dissolved in 0.1 ml. of 10% sulfuric acid, and 2 ml. of concentrated sulfuric acid and 0.1 ml. of a 5% solution of gallic acid in ethanol were added. The mixture was heated on a steam bath for about 15 minutes, but the original rose color of the solution remained unchanged. Under the same conditions, piperonal gave a positive test within 1 or 2 minutes. With piperonal, a greenish color formed which rapidly changed to a deep blue coloration.

REDUCTION TESTS

Approximately 10 mg. of the yellow crystals were boiled with 10 ml. of 5% sodium hydroxide and 1 gram of zinc dust for 1 minute. The hot solution was filtered immediately. A blood red colored filtrate was obtained which formed a red precipitate and a colorless solution in the presence of air.

A small amount of the yellow substance warmed with a solution consisting of ethanol, 1% aqueous sodium hydroxide, and a small quanity of sodium hydrosulfite also formed a blood red solution. This solution when shaken in the air turned to a yellow color. The presence of some ethanol seemed to improve the test because of its solubilizing action.

Both of the above tests gave results similar to the results given by anthraquinone. Under these conditions, anthraquinone is reduced to anthrahydroquinone which dissolves in the alkali to form a blood red solution. Air oxidizes the anthrahydroquinone back to the original quinone which precipitates from solution leaving a colorless supernatent solution.

The reduction product obtained from the action of hydrosulfite on the yellow substance seemed to be oxidized by air back to the starting material. The red material obtained from the zinc dust reduction was probably due to some alkaline degradation caused by the higher strength alkali. The yellow substance dissolved in glacial acetic acid or in 50% acetic acid was not reduced by sulfur dioxide. It was reduced by sodium hydrosulfite when dissolved in acetic acid containing some water.

The yellow substance was slowly decolorized by boiling with a mixture of acetic acid, hydrochloric acid, and tin. The resulting solution was very strongly fluorescent.

The behavior with reducing agents in acid solution is also similar to that of the anthraquinones.

ATTEMPTED ACETYLATION

A previous experiment had indicated that the yellow substance was not acetylated by acetic anhydride and pyridine. In a further attempt to obtain an actate, 46 mg. of the yellow crystals were dissolved in 10 ml. of acetic anhydride by heating under reflux. Freshly fused sodium acetate was added, and the mixture was refluxed for 1 hour. The color gradually darkened during the heating period. The reaction mixture was poured onto ice. After the acetic anhydride had hydrolyzed, the dark colored solution

was filtered to remove a small amount of dark residue, and then was neutralized with sodium bicarbonate. The bright yellow product amounted to 43 mg. and after one crystallization from chloroform melted at 279-281°. There was no depression in the melting point when mixed with the starting material.

In another experiment, 60 mg. of the yellow crystals were dissolved in 10 ml. of hot acetic anhydride, and the solution was cooled to room temperature. A small drop of concentrated sulfuric acid was added. This changed the yellow color to an orange color and caused the formation of a precipitate which dissolved on heating. The mixture was refluxed for one hour, and the reaction mixture was worked up as described above. The yellow product amounted to 60 mg., and was shown to be starting material by the melting point.

After the above experiments were completed, a reference was found to the ease of hydrolysis of the acetate of α-pyridone by water (4). Thus, the acetate of the yellow substance may have been formed in the above reactions, and then hydrolyzed by the water.

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EXTRACTIVES OF YELLOW POPLAR HEARTWOOD
YELLOW PIGMENT I (m. p. 280°)

Project Reports No. 5 and No. 6 described, respectively, a survey study of the extractives of yellow poplar (Liriodendron tulipifera L.) heartwood, and the partial characterization of three crystalline substances, one of which was a yellow pigment (m. p. 280°) containing nitrogen.

This report describes some of the chemical behavior of the yellow Pigment I.

REDUCTION OF PIGMENT I.

Sodium metal acted upon Pigment I in absolute alcohol to form a deep red solution. A small yield of dark red crystalline material was obtained when the reaction mixture was diluted with water, but the crystals were not further investigated. Upon acidification of the reaction mixture with acetic acid, a chocolate-brown precipitate formed. Although this material could not be crystallized, it was partly soluble in dilute hydrochloric acid and was oxidized by chlorine in this medium to an unstable, yellow, crystalline substance, decomp. 166-168°.

The acid-insoluble portion of the above reduction product was readily oxidized in nitric acid with the formation of a colorless crystalline product, decomp. 308-309°.

The pigment was apparently reduced by zinc dust in dilute sulfuric acid, and by lithium aluminum hydride in ether, but no crystalline substances were obtained.

OXIDATION OF PIGMENT I.

Potassium permanganate readily oxidized the pigment in acid solutions. From one reaction mixture a very small amount of an acidic crystalline material was obtained which decomposed at 330-332° with the formation of a crystalline sublimate. The main reaction product failed to crystallize.

Pigment I may have formed a salt with chromic acid, but did not appear to be oxidized with chromic acid or hydrogen peroxide.

ATTEMPTED REACTIONS WITH METHYL IODIDE

Pigment I reacted with methyl iodide to form a dark red, crystalline substance, decomp. 213-215°. The substance was yellow in acid and purple in alkaline solutions. The reaction of Pigment I and methyl iodide in the presence of silver oxide yielded only tarry materials.

REACTIONS OF NITRIC ACID.

Without heating, nitric acid and Pigment I formed a sparingly soluble, orange-colored, crystalline substance, decomp. 260°. When heated with concentrated nitric acid, Pigment I formed a yellow crystalline material, darkened 360° without melting. These substances were not further investigated but the orange crystals may be the nitric acid salt of the pigment, and the yellow substance may be a nitrated or oxidized product.

REACTION WITH SULFUROUS ACID

Pigment I reacted with sulfurous acid in a sealed tube on a steam bath to form a sparingly soluble, orange, crystalline substance, decomp. 280-285°, which may be the acid salt of the pigment.

Exploratory efforts to oxidize or reduce Pigment I, as described above, to difinitive compounds which may aid in determining the structure of the pigment have met with only limited success. Eowever, the reduction with metallic sodium in alcohol followed by an oxidation with chlorine or with nitric acid may yield sufficient material for identification of the fragments. Additional work may include catalytic reduction and zinc dust distillation.

EXPERIMENTAL

REDUCTION OF PIGMENT I WITH SODIUM.

An amount of 116.3 mg. of the yellow crystals was suspended in 20 ml. of absolute ethanol and 818 mg. of sodium metal was added in small pieces over a period of three hours. The yellow substance gradually dissolved to yield a dark-red solution. The flask was not cooled and the temperature reached 40-50°. Water, 70 ml., was added to the reaction mixture whereupon a small amount of material precipitated which was composed of burs of dark red needles, visible under the microscope (300x). The mixture was filtered, the filtrate was acidified with acetic acid which yielded a flocculent, chocolate-brown precipitate which was collected by centrifugation. The product was partially soluble in 95% ethanol but could not be crystallized from that solvent. Some undissolved material was removed from the solution by filtration. The filtrate was acidified with 25 ml. 0.5N hydrochloric acid, and the residue was partially dissolved in another portion of 0.5N acid. Sodium hypochlorite (laundry bleach) was added dropwise to each until the red solutions had changed to deep yellow and a flocculent, yellow precipitate formed.

The solutions were extracted twice with chloroform which yielded a yellow crystalline solid upon evaporation of the solvent. After crystallization from 95% ethanol, the product from the alcohol-soluble material weighed 22.9 mg., decomp. with gas evolution at 166-168°, and the product from the residue was dark colored, weighed 17.4 mg. and decomp. with gas evolution at 160-162°.

The above residue, which was insoluble in 0.5N hydrochloric acid, was heated with concentrated nitric acid until nitrogen dioxide was no longer evolved (about two hours). The solution was diluted with water and was partially neutralized with sodium bicarbonate whereupon a crystalline precipitate formed. The buff crystals were filtered, washed with water, and dried; yield 18.4 mg., decomp. 308-309°. A test for nitrite or nitrate groups with diphenylamine in concentrated sulfuric acid was negative. The crystals dissolved in dilute aqueous alkali and crystallized in colorless needles upon acidification.

ATTEMPTED REDUCTION WITH ZINC IN SULFURIC ACID.

A few milligrams of Pigment I was dissolved in hot 10% sulfuric and zinc dust was added portionwise. The solution was nearly colorless after ten minutes and was strongly, light blue fluorescent. The solution was neutralized with sodium bicarbonate and extracted with chloroform which removed a greenish-yellow material that tended to darken on exposure to air.

ATTEMPTED REDUCTION WITH LITHIUM ALUMINUM HYDRIDE.

A few milligrams of Pigment I was treated with an excess of lithium aluminum hydride. After acidification with acitic acid, the light yellow product was soluble in ether. A paper chromatogram showed the presence of some starting material along with a faster moving spot of a colorless but blue fluorescent material.

OXIDATION OF PIGMENT I WITH PERMANGANATE.
A.-AQUEOUS POTASSIUM PERMANGANATE.

Pigment I, 55 mg., was suspended in 10 ml. of water and heated on a steam bath. Potassium permanganate, 80 mg., was added in five portions over a period of eight hours. The residual manganese dioxide was removed by filtration and washed with water. The combined filtrate and washings were acidified with hydrochloric acid to Congo Red paper, and extracted with chloroform and ether. Only a small amount of material was extracted from the aqueous solution. The manganese dioxide residue was suspended in water, acidified with acetic acid and treated with sodium bisulfite. A yellow substance remained; yield, 28.7 mg. (52%), m. p. (decomp.) 282-3°, and was apparently starting material.

In another experiment 26.1 mg. of Pigment I was oxidized with 6 ml. of 5% permanganate added portionwise. The odor of hydrogen cyanide was quite strong toward the end of the reaction. Upon cooling and discharging of the manganese dioxide with bisulfite, no precipitate formed in the nearly colorless solution, and it was discarded.

B. POTASSIUM PERMANGANATE IN SULFURIC ACID.

Based on a qualitative experiment, 25.8 mg. of Pigment I was dissolved in 5 ml. of hot 10% sulfuric acid. Aqueous potassium permanganate (6%) was added dropwise to the hot solution until the pink color of permanganate persisted for 1-2 minutes. The reaction mixture was cooled and the black precipitate of manganese dioxide was discharged with a minimum of sodium bisulfite. A fine, buff-colored crystalline precipitate was present in the light yellow colored solution. It was removed by filtration, washed with water and dried; yield (estimated), 1-2 mg., darkened at 322°, decomp. 330-2° with the formation of a crystalline sublimate. The crystals were soluble in aqueous sodium hydroxide and insoluble in water and dilute acids, hot or cold, and in chloroform, ether, and 95% ethanol.

The aqueous filtrate was extracted in succession with chloroform, ether, and ethyl acetate:

Extract	Amount recovered, mg.
chloroform	3.9 (three extractions)
ether	4.0
ethyl acetate	18.5
	26.4 mg.

None of the material was crystalline.

The above general procedure was repeated with 23.3 mg. of the pigment dissolved in 5 ml. of 10% sulfuric acid on a steam bath. Aqueous (6%) potassium permanganate, 2.24 ml., was added dropwise. Gas was evolved which had a slight odor of hydrogen cyanide. Upon cooling and treatment with bisulfite a small amount of crystalline precipitate was present.

In other experiments, the permanganate was added very slowly or all at once, but no improvement in the reaction was observed.

C.-POTASSIUM PERMANGANATE IN ACETIC ACID.

Pigment I, 26.8 mg., was dissolved in strong acetic acid (20% aqueous) and 2.5 ml. of 6% permanganate was added portionwise over a period of one hour at room temperature. The reaction mixture was diluted with water, the brown color was discharged with bisulfite, and the solution was extracted with chloroform. Evaporation of the chloroform left a very small amount of partly crystalline yellow material. Neutralization of the aqueous solution with sodium bicarbonate followed by extraction with chloroform resulted in the recovery of a small amount of starting material.

ATTEMPTED OXIDATION WITH CHROMIC ACID

Pigment I, 3-5 mg., was dissolved in mixture of 1 ml. glacial acetic acid and two drops of water after heating on a steam bath. After

cooling, a solution of chromic acid in acetic acid was added dropwise. A cloudiness developed and finally a dense precipitate of small particles formed which did not appear to be crystalline at 300x magnification.

ATTEMPTED OXIDATION WITH HYDROGEN PEROXIDE

Pigment I did not appear to be bleached or oxidized by hydrogen peroxide in dilute sulfuric acid.

ACTION OF METHYL IODIDE

Pigment I, 10-15 mg., was sealed in a small tube with ca. 1 ml. of methyl iodide. The tube was enclosed in a wire screen guard and was heated in a steam bath for one hour. The yellow crystals gradually dissolved and a red crystalline product formed a slush in the tube. The product, decomp. 213-215°, was somewhat soluble in water to form a dark red solution, in acid the solution was yellow, and in sodium hydroxide it was purple. It was not uniformly soluble in chloroform.

In another experiment, Pigment I was heated as above with methyl iodide and silver oxide. A chloroform extract yielded only tarry material which was not extracted from the chloroform with aqueous hydrochloric acid.

ACTION OF NITRIC ACID

An amount of 10-15 mg. of Pigment I was dissolved in ca. 1 ml. of concentrated nitric acid at room temperature to form a bright orange-red

solution. After standing overnight, the addition of 4-5 volumes of water caused the formation of an orange, crystalline precipitate. The precipitate was filtered, washed with water, and dried; it sintered at 245-250°, decomp. 260 to a black liquid with gas evolution.

The experiment was repeated except that the solution was heated on a steam bath for one hour. No apparent change had taken place, and the addition of 5-6 volumes of water precipitated only a small amount of orange-colored material. But after standing for an hour, light yellow needles slowly formed; 7.6 mg. from 10-15 mg. of starting material, darkened at 280° and did not melt below 360° (the limit of the oil bath). The product was negative in the diphenylamine reagent.

ACTION OF SULFUROUS ACID.

Pigment I, 10-15 mg., was sealed in a small tube with 2 ml. of 4-5% aqueous sulfurous acid. The tube was heated on a steam bath for one hour during which the yellow crystals dissolved and an orange-colored crystalline precipitate formed a slush in the tube. The crystals were removed by filtration, washed with water, and dried; product darkened at 270°, decomp. 280-5°.

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THE EXTRACTIVES OF YELLOW POPLAR

Project Report No. 5 described a survey study of the extractives of yellow poplar (Liriodendron tulipifera L.) heartwood in which a yellow coloring matter was isolated by chromatographic means. The present report describes an improved isolation of the yellow material and the isolation and partial characterization of two colorless crystalline materials.

One of the colorless products appears to be a cyclitol or a mixture of cyclitols, although definite evidence of its cyclitols nature has not been obtained. The second colorless product gives a positive Mäule test and contains a high percentage of methoxyl. The yellow coloring matter contains nitrogen, and is extracted from benzene or chloroform solution with dilute hydrochloric acid. This material crystallizes from chloroform in bright yellow needles.

J. U. Lloyd, American Druggist 40, No. 6:101 (1886), described the "characteristic" coloring matter of yellow poplar, and stated that fresh root bark contains considerable amounts of the material. Although, the material described by Lloyd had quite different properties from the yellow material isolated in this work, fresh roots were examined in the hope that larger amounts of the crystalline material might be found. However, the small roots available to us did not contain the yellow crystalline material which has been isolated from the heartwood.

EXPERIMENTAL

EXPLORATORY STUDIES

Some early experiments had been carried out before the work described in Project Report No. 5. These experiments are reported in this section.

95% Ethanol Extraction

A few grams of yellow poplar heartwood sawdust were extracted in a Soxhlet extractor with 95% ethanol, and the extract was adsorbed on a No. 1 column of magnesia (USP, heavy, Merck)-Celite, 5:1 by weight. The chromatogram was developed with 95% ethanol, and the effluent was collected until a leading yellow band was washed from the column. No attempt was made to recover material from the column. The effluent was evaporated under a current of air, and colorless crystals deposited. The crystals were washed with alcohol and dried; m.p. 221-3°C.

In a somewhat larger scale separation, the water-soluble crystals were separated from non-crystalline yellow material by filtration of an aqueous solution. The filtrate was concentrated to a small volume, and absolute ethanol added. The white crystals which formed were filtered, washed with 95% ethanol, and dried; m.p. 222-3°.

In a still larger scale separation, 7.8 grams of extract obtained from 375 grams of airdry sawdust were dissolved in approximately 50 ml. of 95% ethanol, 2-3 grams of Celite were suspended in the solution, and 175 ml. of water were added with efficient stirring and moderate heating. After cooling, the slurry was filtered on a sintered glass funnel, and the residue was

washed with water. The combined filtrate and washings were concentrated under reduced pressure. A heavy tar deposited on the walls of the flask, and was separated from the supernatent solution. Further concentration of the solution resulted in the formation of a crude crystalline material; m.p. 210-214°. After three crystallizations from aqueous ethanol, 183 mg. of crystalline material was obtained which still melted at 210-14°; this sample was used for analysis. Although the melting point is lower, this material appears to be the same as the crystals obtained previously melting at 222-23°.

A 0.358-gram sample of the crystalline material was acetylated by heating on a steam bath for 8 hours with 3 ml. of pyridine and 5 ml. of acetic anhydride. The crystals dissolved in the acetylation mixture after heating for about 5 hours. After standing overnight at room temperature, the reaction mixture was poured onto ice water, and the acetate was extracted with chloroform. Evaporation of the chloroform gave a colorless sirup which eventually crystallized. After recrystallization from 95% ethanol, the product partially melted at 132°, and then melted completely at 180-88°.

50% Ethanol Extraction, Water Soluble Fraction

Yellow poplar heartwood sawdust (449 grams) was suspended in 3 liters of 50% ethanol. The suspension was transferred to a large percolating tube, and the sawdust was leached with 5 liters of hot solvent. The combined extract was concentrated in a circulating laboratory evaporator, and was separated into a water-soluble and a

dark-brown water-insoluble fraction. A second lot (686 grams) of sawdust was suspended in 4 liters of 50% ethanol, and was leached with 8 liters of hot solvent.

The water-soluble fraction contained material which corresponded on paper chromatograms to the 222° melting crystals obtained from the 95% ethanol extract. Both were similar to meso-inositol as indicated by a permanganate periodate spray. This chromatographic behavior along with the melting point behavior of the acetate suggest that the water-soluble colorless crystalline material may consist of a cyclitol. However, definite evidence for a cyclitol has not been obtained, but the data do indicate that the material is some type of polyhydric alcohol.

FURTHER STUDIES

Water-Insoluble Fraction of 50% Ethanol Extract

The water-insoluble fraction of the 50% ethanol extract was dissolved in 95% ethanol, and the solution was uniformly adsorbed on 300-400 grams of Celite. After air drying, the mixture was stirred for 30 minutes with 1.5 liters of 50:1 benzene-ethanol. The mixture was filtered, and the residue washed with fresh solvent. The solution was concentrated to approximately 500 ml., and half of the concentrate was transferred to a No. 4 column of alumina (Fischer Scientific Co., adsorption grade) with 200 ml. of benzene. (The other half of the concentrate was treated in the same manner on a fresh column). The column was developed with 500 ml. of 20:1 petroleum ether-ethanol, 1000 ml. of 9:1 petroleum ether-ethanol, and finally with 500 ml. of 4:1 petroleum ethan-ethanol. Five fractions of effluent were collected:

Fraction 1 consisted mainly of benzene, and contained a small amount of wax, but no fluorescent materials.

Fraction 2 contained some bright blue-fluorescent material. Colorless crystals formed which were washed with 95% ethanol; m.p. (sublimes) 267-275°. Subsequent isolations yielded none of this material and no further work was done on the material available.

Most of the solids in this fraction remained in the mother liquor, which on partial evaporation turned to a heavy slush of colorless crystals. These were washed with ether and petroleum ether; m.p. 102-112°. This material was insoluble in water, and gave a strong Maule test characteristic of materials containing syringyl groupings. It did not give an immediate test with ferric ferricyanide, and probably does not contain free phenolic hydroxyl groups.

Fraction 3 consisted of a small amount of dark-colored material and was discarded.

Fraction 4 contained the main part of the bright-yellow substance, and on partial evaporation formed yellow crystals. After recrystallization, the yellow crystals darkened at 275° and melted at 276-79°. This substance is the yellow coloring matter described in Project Report No. 5.

Fraction 5 contained only a small amount of material, and was discarded.

Ethanol-Benzene (1:10) Extraction

Since 1:10 ethanol-benzene is a good solvent for the extraction of the yellow substance (Project Report No. 5), additional quantities of heartwood sawdust were extracted with this solvent in a Soxhlet apparatus. The extract from 365 grams (346 grams dry basis) of sawdust was concentrated to about one third of the original volume, and the concentrate was agitated by hand with 65 grams of alumina for 30 minutes. The mixture was transferred to a No. 4 tube containing 85 grams of alumina, and the column was developed with 150-200 ml. of benzene, 500 ml. of 30:1 petroleum ether-ethanol, 400 ml. of 3:1 petroleum ether-ethanol, and finally with 250 ml. of 2:1 petroleum ether-ethanol. Three fractions were collected:

Fraction 1 (1000-1200 ml.) was mainly benzene and was colorless.

Fraction 2 (550 ml.) was light yellow in color and contained blue fluorescent material. On standing, bundles of prisms formed; m.p. 105-113°. This material gave a strong Maule test, and is apparently the same as the second lot of crystals obtained from fraction 2 of the water-insoluble part of the 50% ethanol extract.

Fraction 3 (less than 500 ml.) contained the bright yellow substance. Evaporation of the solvent gave yellow crystals which were washed with 95% ethanol; m.p. 275-77°.

The extract from a second lot of sawdust (374 grams) was treated in the above manner, and the final fractions were combined with the above fractions.

Chloroform Extraction

During the analytical part of this study, Mr. Willemsen found that the yellow substance could be crystallized from chloroform. Accordingly, chloroform was investigated as a possible solvent for the extraction of the wood. For this purpose, an 8-gram sample of yellow poplar heartwood was extracted in a small Soxhlet successively with chloroform, 1:10 absolute ethanol-chloroform, and 1:10 absolute ethanol-benzene for 4 to 5-hour periods. The yields of extract were respectively 1.5, 1.8, and 0.4%. Paper chromatography indicated that all of the extracts contained some of the yellow substance, but the amount in the ethanol-benzene extract was small. This extract consisted chiefly of a light-colored amorphous material which melted at about 85-90°. The two chloroform extracts contained material with a pink fluorescence, and the two ethanol extracts contained material with a blue fluorescence. Since chloroform alone did not remove all of the yellow substance, its use as an extracting solvent was not investigated further.

Acid Separation

An attempted acid hydrolysis described in Project Report No. 5 indicated that "the acid treatment converted the yellow material to an acid-soluble material which was more deeply colored than the original material." Originally it was believed that the hot acid had caused some type od decomposition, but the results could have been due to the formation of a water soluble salt with hydrochloric acid. In order to test this possibility, a small amount of a benzene solution of the ethanol-benzene (1:10) extract was extracted with 10% hydrochloric acid. The resulting

red colored acid solution was carefully neutralized with solid sodium bicarbonate. The yellow colored neutralized solution was then extracted twice with benzene and once with chloroform. Evaporation of the benzene gave a clean yellow solid, and evaporation of the chloroform gave a darker yellow-colored solid. The typical crystals of the yellow substance were obtained from both solids by crystallization from chloroform. Thus, the yellow substance appears to be a basic material which forms a water-soluble hydrochloride.

In a larger scale separation, 4.4 grams of ethanol-benzene (1:10) extract (from 345 grams of wood, dry basis) were dissolved in chloroform, and the chloroform solution was washed with 50 ml. of water. Evaporation of the aqueous solution gave 0.07 gram of material which was not investigated further. The chloroform solution was next extracted four times with 25 ml. portions of 10% hydrochloric acid. The combined acid solutions were combined, washed once with chloroform and then filtered to remove some sludge. The filtered solution was covered with a layer of benzene, and was neutralized with solid sodium bicarbonate. The neutral solution was extracted four times with 100 ml. portions of benzene and once with 100 ml. of chloroform. Evaporation of the combined benzene solutions gave 0.17 gram of yellow material which was recrystallized from 10 ml. of chloroform and gave 0.09 gram of material melting at 278°. An additional 0.05 gram of material were obtained by evaporating the mother liquor to about 5 ml. A second recrystallization increased the melting point to 280°. Evaporation of the chloroform extract gave 0.02 gram of material which was not investigated further. The total yield of crystalline material was 0.04% based on the wood.

In another separation, the ethanol-benzene (1:10) extract from 357 grams (dry basis) of wood was evaporated to 40% of the original volume, and was extracted directly with 6-50 ml. portions of 10% hydrochloric acid. The resulting acid solution stood over the week end, and some insoluble material formed on the bottom of the beaker. After neutralization with sodium bicarbonate, the solution was extracted five times with 100 ml. portions of benzene and three times with chloroform. Evaporation gave 0.78 gram of material from the benzene extract and 0.27 gram from the chloroform extract. The benzene extracted material was crystallized from 10 ml. of chloroform, and the chloroform extracted material was crystallized from 7 ml. of chloroform. The two resulting lots of crystals were combined and amounted to 0.1 gram. Evaporation of the mother liquor to about 5 ml. gave an additional 0.05 gram of crystals. In this case, the total yield of crystalline material was 0.03%.

Analytical Data

Various preparations of three crystalline material were combined and were recrystallized. Analytical data for the purified materials are given in Table I and absorption data are reported in Table II.

The polyhydric alcohol was recrystallized from dilute ethanol until the melting point was constant. The carbon and hydrogen data do not agree with any specific polyhydric alcohol, but the carbon to hydrogen ratio does suggest that the material is a cyclitol. Very likely this material consists of a mixture of at least two cyclitols which differ in the number of oxygens present. As expected for a cyclitol, this material has practically no absorbence in the ultraviolet.

The colorless product which gave the positive Maule test was recrystallized from 95% ethanol, but the recrystallized product still melted over the range of 107-113°. Thus the material which was analyzed was probably a mixture of at least two substances. Attempts to derive a possible structure from the analytical data also suggested that the material is a mixture. The high methoxyl content indicates that an average of two methoxyl groups are present per each eight to nine carbon atoms. This material had the high absorbence in the low range of the ultraviolet spectrum typical of aromatic compounds, but the absorbency above 240 mmu was rather low.

The yellow coloring matter was recrystallized from chloroform before analysis. Again the analytical data do not agree with any relatively simple structure. Thus it seems likely that the material was impure. Attempts to determine the molecular weight of this material were unsuccessful because of a lack of suitable solubility. This material showed maxima in the absorption spectrum at 247, 268, 310, and 408 mmu, and minima at 233, 260, 292, and 342 mmu.

TABLE I

ANALYTICAL DATA

Sample		Polyhydric Alcohol	"Maule Positive Substance	Yellow Pigment
Carbon, %		45.14	64.29	72.45
		45.04	64.68	72.58
	Av.	45.09	64.48	72.50
Hydrogen, %		7.61	7.01	3.35
		7.63	6.85	3.35
	Av.	7.62	6.93	3.35
Nitrogen (Dumas), %		--	--	4.78
				4.78
	Av.			4.78
Methoxyl, %		--	40.0	0.0
			40.7	
	Av.		40.4	
Molecular weight:				
Rast method		--	330	--
Pregl direct method		--	370	
Pregl indirect method		--	338; 292	

TABLE II

ABSORPTION DATA

Sample	Polyhydric Alcohol	"Maule" Positive Substance	Yellow Pigment
Wavelength, mmu.	Specific Extinction	Specific Extinction	Specific Extinction
220	0.5	57.6	85.5
225	0.4	45.9	87.2
230	0.3	41.5	80.9
233	--	--	73.4
240	0.0	28.6	83.7
247	--	--	95.6
250	--	6.8	89.1
260	0.1	3.6	69.4
268	--	--	79.9
270	--	5.0	78.4
280	0.1	3.8	53.8
292	--	--	20.4
300	0.1	0.5	23.0
310	--	--	25.0
320	0.0	0.1	20.3
342	--	--	9.2
360	0.0	0.0	16.4
380	0.0	0.0	26.8
400	0.0	0.0	36.9
404	--	--	39.0
406	--	--	38.5
408	--	--	39.2
410	--	--	39.0
414	--	--	39.0
418	--	--	39.6
420	--	--	37.4
430	--	--	24.6
440	--	--	11.0
450	--	--	3.4
460	--	--	1.3
480	--	--	0.2
500	--	--	0.1
540	--	--	0.2
560	--	--	0.1
600	--	--	0.1
650	--	--	0.1
700	--	--	0.0

(Concentration: 0.02 g./l. in 95% ethanol)

Examination of Roots

Fresh yellow poplar roots were obtained January 11, 1956 through the courtesy of Jack Leech of West Virginia Pulp and Paper Company. These were small roots, the maximum diameter being about one half inch. The bark was peeled from the roots, and the separated root bark and root wood were extracted first with benzene and then with ethanol in Soxhlet extractors. Some benzene-insoluble material collected in the flasks during the benzene extractions, and likewise some ethanol-insoluble material was removed in the ethanol extractions. The yield of the different extracts are tabulated in Table III.

TABLE III

EXTRACTION OF YELLOW POPLAR ROOTS

(Yields expressed as percentages of the original sample, dry basis)

Sample	Root Bark	Root Wood
Benzene extract:		
Benzene-soluble, %	6.5	0.8
Benzene-insoluble, %	3.8	1.5
Ethanol extract:		
Ethanol-soluble, %	7.9	4.7
Ethanol-insoluble, %	0.2	0.4
Total extract, %	18.4	7.4

Paper chromatography with butanol-pyridine-water 6:4:3 showed that none of the root extracts contained the yellow pigment which had been isolated from the heartwood. The red-colored benzene extracts of the root bark and of root wood contained material which gave a positive test with periodate spray (Rf 0.87). The benzene extract of the root wood gave a blue spot with ferric ferricyanide spray at Rf 0.84. The ethanol extracts

of both the bark and the root wood gave glucose and light fructose spots with aniline hydrogen phthalate spray.

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THE EXTRACTIVES OF YELLOW POPLAR HEARTWOOD

Yellow poplar (Liriodendron tulipifera L.) is used to a certain extent as a pulpwood in the Appalachian area. Sulfite pulp prepared from this species is said to be of poor color and difficult to bleach [Wells, S. D., and Rue, J. D., U. S. Dept. Agr., Dept. Bull. 1485. May, 1927. 101 p.]. Difficulty has also been experienced in bleaching yellow poplar groundwood [Kingsbury, Ralph M., Simmonds, Forrest A., and Lewis, Earle S., Tappi 32:273 (1949)]. Groundwood stored in the moist condition at room temperature for several days could be bleached with alkaline hypochlorite in the normal manner, but the same groundwood stored at 5°C. did not respond to the bleaching procedure. Likewise fresh groundwood extracted with alcohol (which removed a yellow-colored material) could be bleached. These results suggest that the wood contains some coloring matter which can be extracted with organic solvents. The results obtained on storage suggest that some enzyme action can convert the colored material into substances which do not cause trouble in alkaline peroxide bleaching.

Apparently information on the nature of yellow poplar extractives has not been published. In previous work Mr. Dickey isolated a colorless crystalline material from mixed sapwood and heartwood.

This substance which melted at about 223°C. seemed to be a cyclitol but was not definitely identified. This report describes an orienting study of the extractives of yellow poplar heartwood. A search was made for suitable means of extracting the materials and for separating the extracts into individual components.

Paper chromatography using butanol-pyridine-water 6:4:3 indicates that the heartwood contains several extractives: a yellow coloring matter with an Rf in the range of 0.65 to 0.75, material which reacts rapidly with ferric ferricyanide or permanganate-periodate sprays which is probably phenolic and shows up at Rf 0.75 to 0.90, material giving a Maüle test at Rf about 0.85, materials with low Rf values which react slowly with permanganate-periodate, and several flourescent materials. In addition there appears to be rather large amounts of phlobaphene-like material.

The yellow coloring matter is soluble in benzene and is not extracted from benzene with 1% sodium hydroxide solution. It appears to be slowly decomposed by alkali at room temperature and is completely changed by alkaline saponification. The alkaline decomposition products are brown colored and appear to be of indefinite composition. Light also slowly changes the yellow material to a brownish material. Acid hydrolysis converts the yellow product to a dark colored acid-soluble material. The yellow material shows a bright yellow fluorescence in ultra violet light.

Most of the yellow material is extracted from the wood with benzene, and essentially all of it is extracted by absolute ethanol-benzene

1:10. The latter solvent mixture appears to be especially suited for the extraction of the yellow material. Concentration of the resulting solution to one half volume removes all of the alcohol and precipitates some dark colored material and leaves nearly all of the yellow material in the benzene solution. Aqueous propanol (water-n-propanol 1:3) is a good solvent for complete removal of the extractives from yellow poplar wood. It has been used as the final solvent for extracting ethanol-benzene extracted wood.

EXPERIMENTAL

Several lots of yellow poplar heartwood sawdust prepared previously and stored in an air-dry state were used in this study. The moisture content of the sawdust at the time of the present work was about 6%.

EXTRACTION

Several small scale Soxhlet extractions were made in order to develop procedures for further work, and the results are summarized in this section. The extracts were chromatographed on Whatman No. 1 paper using butanol-pyridine-water (6:4:3) as the developer.

Extraction with water-n-propanol 1:3, (approximately the azeotropic mixture) was very effective in removing the extractives. The water was added to the dry sawdust and the wood allowed to soak for 1-2 hours. The propanol was then added to the boiling flask and the

extraction started. This procedure, which is referred to as aqueous propanol extraction, gave a rapid removal of the colored materials (1 to 2 hours) and extracted 5 to 6% material based on the dry wood weight. Although, the aqueous propanol removed the colored materials from the wood very rapidly, the resulting extract was a complicated mixture of substances which appeared difficult to work with. Consequently, a search was made for more selective solvents.

Extraction with petroleum ether removed from 0.2 to 0.4% of colorless materials. Although preextraction with petroleum ether has some advantage, the chromatographic procedures described later seem to remove the same materials. Consequently the extraction with petroleum ether was omitted in the later work. A benzene extraction of the petroleum ether-extracted wood removes a mixture of colored and colorless materials amounting to 1, to 1.5% of the wood. Aqueous propanol extraction of the benzene-extracted wood removed about 2% of a dark colored extract.

One of the materials extracted with benzene gave a strong yellow spot of Rf about 0.75 on the paper chromatogram. This material has high tinctorial properties, and probably is an important factor in the yellow poplar bleaching problem. All of this material was not extracted with benzene, and therefore mixtures of alcohols and benzene were tested. All of the yellow material could be extracted with mixtures of ethanol and benzene or with a propanol-benzene. Absolute ethanol-benzene (1:10) was selected as the best of the mixtures tested. This rapidly removed all of the yellow material without removing large

amounts of a dark colored phlobaphene-like material which is present. In addition, the alcohol can easily be removed from this mixture by distillation. Concentration (at reduced pressure) of the ethanol-benzene extract to one half volume results in a benzene solution containing practically all of the yellow material (along with other materials), and results in the separation of small amounts of benzene-insoluble materials. The latter apparently consist of some colorless material and a dark colored phlobaphene-like material. A subsequent extraction of the wood with aqueous propanol removes larger amounts of these materials.

The results from a larger scale extraction were as follows: Approximately 345 grams (dry basis) yellow poplar heartwood sawdust were extracted in a Soxhlet-type apparatus for 3 hours with 3100 ml. of 1:10 absolute ethanol-benzene. The wood meal was air-dried and then extracted for 4 hours with aqueous propanol. The ethanol-benzene extract was concentrated to 500 ml. and the dark colored insoluble material which formed was separated by filtering. Aliquots of the benzene soluble fraction of the ethanol-benzene extract and of the aqueous propanol extract were evaporated in order to estimate the yields. The yields based on the dry weight of the wood were: benzene-soluble fraction 1.3%, benzene-insoluble fraction of the ethanol-benzene extract 0.4%, aqueous propanol extract 2.6%, total 4.3%. Paper chromatography indicated that the benzene-soluble fraction contained nearly all of the yellow coloring material, and the largest amount of the material which gives a Maüle test. In addition there were various

fluorescent materials, and appreciable amounts of materials which give tests with ferric ferricyanide, and pergmanganate-periodate reagent. The benzene-insoluble fraction and the aqueous propanol extract both contained materials which gave tests with ferric ferricyanide and with permanganate-periodate in the range of Rf 0.77 to 0.86. Both gave slight Mäule tests at about Rf 0.86. The benzene-insoluble fraction gave a permanganate-periodate test at Rf 0.35, and the aqueous-propanol extract gave permanganate-periodate tests at Rf 0.22 and 0.08. The latter spot is probably due to the cyclitol previously isolated by Mr. Dickey.

COLUMN CHROMATOGRAPHY

Several attempts were made to separate the constituents of the benzene (or ethanol-benzene) extracts by means of chromatography. Acid washed Magnesol, alumina, and silicic acid were tested as adsorbents. The best results were obtained with alumina (Fischer Scientific Co. alumina, adsorption, Cat. No. A-540). In the initial experiments with acid washed Magnesol, the column was developed with mixtures of absolute ethanol and benzene, but mixtures of absolute ethanol and petroleum ether (30-75° b.p.) appeared to be better developers and were used in the latter work.

A typical separation was carried out as follows: Yellow poplar heartwood sawdust (30.1 g.) was extracted with absolute ethanol-benzene (1:10) for 1 hour, and the extract was concentrated under reduced pressure to 48 ml. A small amount of colorless

material and considerable dark colored material separated. Absolute ethanol (1 ml.) was added, and the solution mixed with the insoluble material. The solution was filtered, and the residue washed with 25 ml. of 1:25 ethanol-benzene. The combined solutions were poured onto a No. 3 column of alumina. The column was washed first with 25 ml. of benzene, followed by 100 ml. of petroleum ether, and then was developed with absolute ethanol-petroleum ether, and then was developed with absolute ethanol-petroleum ether. Fractions of the effluent were collected as follows: The first fraction consisted of colorless material which appeared to be similar to the petroleum ether-soluble material. The second fraction consisted of a narrow blue fluorescent band with an irregular shape. This fraction was colorless on the column but formed a brownish yellow solution. The third fraction was an intermediate fraction taken after all of the blue fluorescent material was removed and before the yellow band came off of the column. The fourth fraction was a visible yellow band with a yellow fluorescence which formed a yellow solution. The final fraction consisted of dark colored material which formed a brown solution. The various fractions were evaporated to dryness under reduced pressure, and the yields and developing solvent are indicated in Table I.

TABLE I

ALUMINA SEPARATION

Fraction	Yield, g.	Developer
1. colorless	0.036	1:20*
2. blue fluorescence	0.175	1:20*
3. intermediate	0.017	1:20*
4. yellow	0.027	1:10*
5. brown	0.009	1:1**

*absolute ethanol-petroleum ether

**absolute ethanol-benzene

All of the fractions described above were mixtures, but Mr. Dickey has obtained crystalline materials from similar fractions (this will be described in a later report).

SODIUM HYDROXIDE TREATMENTS

A benzene extract obtained from 8.7 grams of the yellow poplar heartwood was extracted with 3-25 ml. portions of 1% sodium hydroxide which contained 0.5% sodium hydrosulfite (to minimize any possible oxidation). The benzene solution was then washed with 2 portions of water, partially dried over anhydrous sodium sulfate, and evaporated to yield 0.038 gram of neutral material. The alkaline aqueous layer which was yellow colored was acidified with dilute sulfuric acid, and the acidified solution was extracted with benzene. This benzene solution on drying and evaporation yielded 0.030 gram of material which is

referred to as the first acidic fraction. On acidification and extraction with benzene some insoluble sludge formed. In an effort to extract all of the acidified material, the acid solution was next extracted with a mixture of n-propanol-benzene (1:5). This yielded 0.008 gram of a second acidic fraction.

Paper chromatography of the three fractions gave the following results: The typical yellow spot (Rf 0.76) was present in the neutral fraction, but was absent in the two acidic fractions. The neutral fraction also had a pink fluorescence at Rf 0.71. Both the neutral fraction and the second acidic fraction gave a blue fluorescence and a phenol test at Rf about 0.85. The first acidic fraction showed a blue fluorescence and a phenol test at an Rf of 0.76. These results indicate that the yellow substance is a neutral material.

In another experiment, 58.7 grams (55.2 grams dry basis) yellow poplar heartwood were first extracted with petroleum ether which removed 0.2% of material. The wood was next extracted with 1:10 ethanol-benzene. This removed an additional 2.2% of material. The ethanol-benzene solution was extracted with 4-25 ml. portions of 1% sodium hydroxide (without any hydrosulfite). During the latter extractions, the lower part of the aqueous layer was colorless while the upper part was dark colored. This suggested that the alkali was slowly decomposing some of the neutral material to a dark-colored alkali-soluble material. In

this experiment, the acidic fraction was extracted from the acidified aqueous solution with 1:1 ethyl acetate-benzene. Some reddish insoluble material formed in this extraction which was insoluble in butanol, propanol, and ethanol. In this case, the neutral fraction amounted to 1% of the wood, and the acidic fraction to 0.7%, with a loss of 0.6% during the alkali separation.

Approximately 0.1 gram of solids obtained by evaporation of an ethanol-benzene (1:10) extract was saponified by refluxing with 10 ml. of 0.5 N alcoholic potassium hydroxide. The alkaline reaction mixture was acidified and extracted with benzene followed by 1:10 n-propanol-benzene. Evaporation of the combined solvent gave only 0.4 gram of material. This material on a paper chromatogram gave: a colorless blue fluorescence at Rf 0.56, a pink-yellow fluorescence at Rf 0.71, a visible yellow-brown spot at Rf 0.79 which adsorbed ultraviolet light and gave a strong phenol test, and a visible brown streak of Rf about 0.83 had a blue fluorescence and gave a phenol test. These results suggested that the saponification procedure converted the yellow material to phenolic materials which were brown colored. In addition to any possible saponification, the alkali seems to produce indefinite decomposition reactions.

ACID HYDROLYSIS

Approximately 30 mg. of the yellow material which had been separated by means of an alumina column was dissolved in a

small amount of hot ethanol. (A small amount was spotted on paper for a later chromatogram). The solution was diluted with 50 ml. of 2% hydrochloric acid, and the mixture refluxed for 2 hours. After cooling, the acid solution was extracted with benzene which removed only 3 mg. of benzene soluble-material. The extracted aqueous solution was dark red in color (darker than before hydrolysis), and on evaporation at room temperature yielded 28 mg. of dark-colored material. The two fractions along with the original solution were chromatographed using butanol-pyridine-water (6:4:3) as the developing solvent. The original solution before hydrolysis showed the typical visible spot with a yellow fluorescence at Rf 0.75, and also a pink fluorescence at Rf 0.53. The benzene-soluble fraction showed only 2 faint fluorescent spots at Rf 0.76 and 0.84. The concentration of the material on the paper may have been too low to indicate other spots. The water-soluble fraction gave a visible yellow spot with a green fluorescence at Rf 0.76 and a pink fluorescence at Rf 0.70.

Apparently the acid treatment converted the yellow material to an acid-soluble material which was more deeply colored than the original material.

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PROJECT REPORT FORM

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COOPERATOR Institute
REPORT NO. 1-4
DATE 7/19/50
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ALCOHOL EXTRACTION OF WESTERN RED CEDAR

INTRODUCTION

This report describes the preparation of a 95% ethyl alcohol extract from milled Western Red Cedar grown in the Pacific Northwest and received at the Institute from the U.S. Forest Service in Portland, Oregon.

Extract samples containing a total of 11.3 pounds of extractives were prepared. Based on wood analyses for total alcohol extractives before and after the extraction, this represents a recovery of 82% of the removed extractible material. Total solids removed in the extraction amounted to 86% of initial alcohol soluble material.

Observations that were made during the extraction process indicated that 250 pounds of steam and 5100 gallons of water were consumed for each pound of dry combined extract.

Equipment and procedure involved in the preparation of the extract are described and results are discussed in the individual steps of the process.

GENERAL DISCUSSION OF PROCEDURE AND EQUIPMENT

After the wood had been prepared for the extraction, it was weighed into two batches of 75 pounds and 69.5 pounds respectively.

The extraction vessel was a cylindrical copper tank 24 inches in diameter and 4'0" in depth; supported on trunnions to allow tipping to handle the wood charge. The ends of the vessel were dished, flanged, and fitted with suitable openings for liquor and vapor passage. Solids were retained on a perforated false bottom and #14 mesh screens and a wooden checkerwork was included to alleviate troubles from packing. Solvent was drawn from the base of the extractor through 1 1/2" diameter brass lines to the 1/2 H.P. Cameron Motor-pump, discharged through a vertical coil-in-shell heat exchanger, and returned to the top of the wood charge through a 1" diameter brass line and shower pipes installed inside the vessel head.

Solvent vapors passed through a 2" diameter line to the top of a reflux condenser which is 10 inches in diameter and about 7' 6" high. Losses through vaporization were minimized by return of the condensate to the base of the extractor. This also had the effect of introducing small quantities of fresh solvent into the circulating extract.

Countercurrent extraction procedure was approached in that solvent which had been used in extracting partially spent wood was returned to the extraction cycle as solvent in treating a succeeding fresh wood charge. One 50 gallon stainless steel tank, and another 100 gallon galvanized iron tank were used for intermediate storage of these weak extracts.

When the circulating extract reached a concentration which remained essentially constant, circulation was stopped, and the extract was diverted to the suction of the filter press pump. Standard duck twill filter cloths on the 12" x 12" Shriver filter press were effective in removing suspended material from the extract before it was discharged through a 3/4" brass line to the still for concentration.

The distilling section was operated at reduced pressure as concentration and solvent recovery were carried out. Vapors from the 125 gallon steam-jacketed copper distilling kettle passed through the 8" diameter 20-plate fractionating column, and were condensed in the 8" diameter copper shell-and-tube condenser. Two 1/4 H.P. Micro-Westco pumps returned reflux to the column and to the kettle, and final distillate was collected in 25 gallon copper receivers.

Further solvent recovery was accomplished by direct

steaming of the wood charge after extraction. Residual solvent was driven off to the 10 inch condenser, and the condensate was subsequently concentrated to 95% ethyl alcohol for further use.

Samples of extract and concentrate were transferred to suitable tared containers and submitted for further study.

WOOD PREPARATION

As received for this preparation, the Western Red Cedar consisted of six pieces, 6" - 8" in diameter and about 3 1/2 feet long. These had been tagged and bound together for shipment.

Before the sticks could be barked, it was necessary that the shipping tags and wire staples be removed. A draw-knife was used to complete the bark removal, and the wood was ready to be reduced to a size suitable for efficient extraction.

Initial reduction of the sticks was accomplished with the Carthage Chipper. When the unit had been thoroughly cleaned to prevent other wood varieties being included with the cedar, the sticks were fed into the chipper which reduced them to 3/4"-1" chips.

When it was found that the available hammer mill exerted too great a pulverizing action on the wood, it was decided that the

No. 1 Wiley Mill should be used to reduce the chips to a still smaller size.

The chips were fed manually into the mill and a product closely resembling sawdust was collected in paper-board containers.

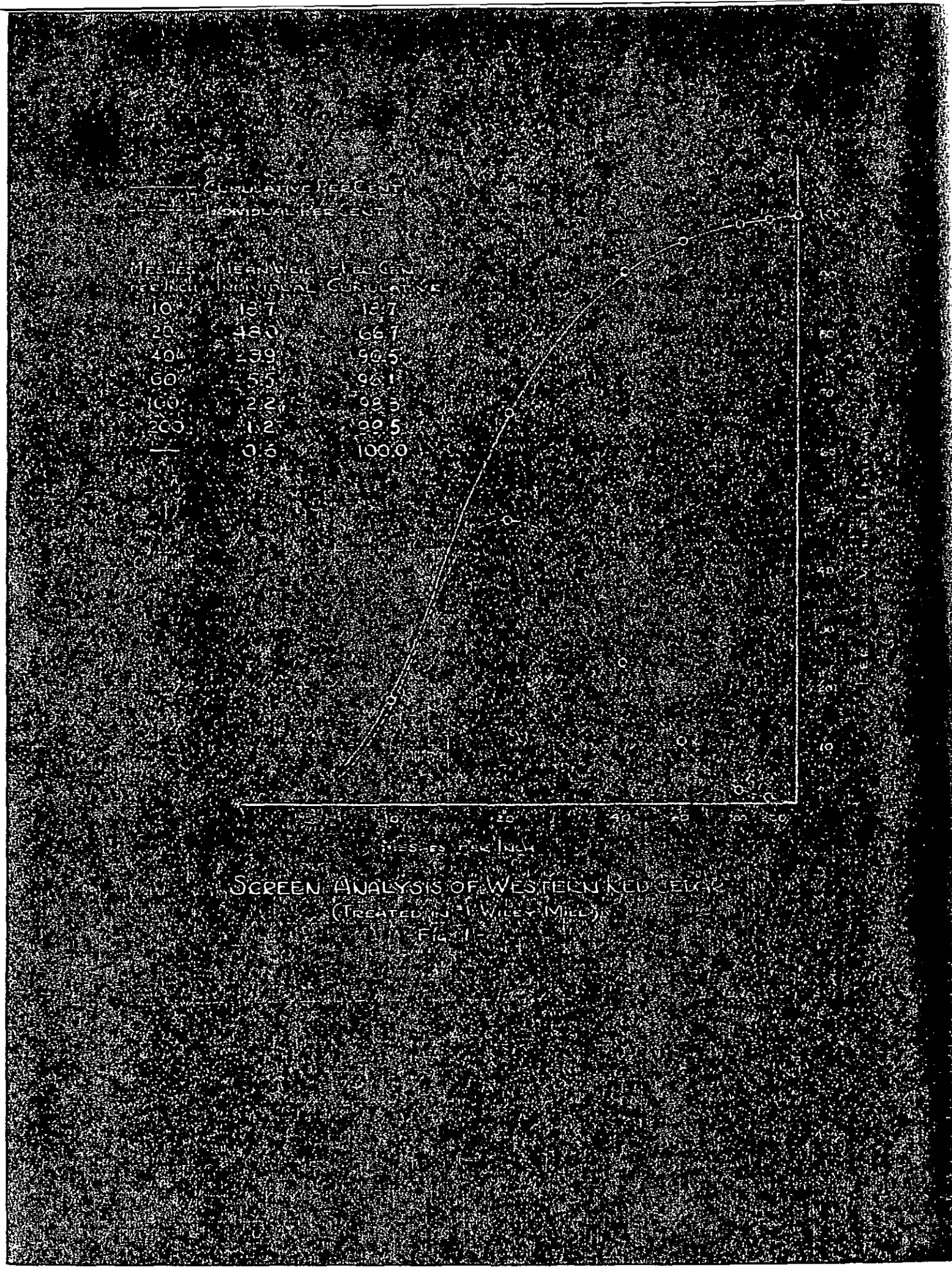
Results of screen analyses are included with this report. Figure I represents the average results from an analysis of each batch which had been prepared for extraction.

TABLE I

SCREEN ANALYSIS OF WESTERN RED CEDAR

Openings (Inches)	Meshes per Inch	Material Retained I (gm.)	I (%)	II. (gm.)	II. (%)	Per Cent Cumulative Weights I	II
.0650	10	27.2	15.0	54.15	22.4	15.0	22.4
.0331	20	85.6	47.1	117.8	48.8	62.1	71.2
—	40	48.8	26.9	50.4	20.9	89.0	92.1
.0098	60	12.0	6.6	10.95	4.5	95.6	96.6
.0059	100	4.6	2.5	4.6	1.9	98.1	98.5
.0029	200	2.3	1.3	2.4	1.0	99.4	99.5
—	-	1.1	0.6	1.1	0.5	100.0	100.0

It is evident that, as charged to the extractor, 90% of the material was retained on 40 mesh or coarser screens. It was not felt that the small percentage of fines (only 5% less than 60 mesh)



Screen Analysis of Western Red Cedar
(Treated in "I. Wiley" Mill)
Fig. 1.

would cause much difficulty by packing. Small amounts that were carried over with the extract were removed in the filter press.

EXTRACTION

Since the preparation of extract for surpassed procedure study in importance, observed data during the extraction was meagre, and not particularly significant. Table II represents a summary of data which was recorded as the extraction progressed.

TABLE II

SUMMARY OF EXTRACTION DATA

Wood Charge		1	2
Weight of Wood Charge (lbs.)		75.0	69.5
Total Extractives (lbs.)		8.34	7.76
Weight of 95% Alcohol (lbs.)		937.0	1115.0
Extraction time (hrs.)	(1)	93.0	22.5
	(2)	2.0	3.0
	(3)	2.5	22.0
	(4)	1.5	1.5
Solids Content of Final Extract (%)		0.08	0.08
Extraction Temperature (° C.)		30°-78°	78°
Residual Alcohol Extractives (lbs.)		1.41	0.85
Alcohol Extractives Removed (lbs.)		13.84	
" " "	(%)	86.1	

Solvent was circulated at a moderate rate through the heat exchanger, and back to the top of the extractor.

No definite operating schedule was followed, since piping adjustments and maintenance were time-consuming. From time to time, however, representative samples were withdrawn from the circulating line for solids determinations. As the concentration became essentially constant, the extract was drawn off, and fresh solvent was added until the final extract showed a solids content of less than 0.1% (Table II). There is no doubt that further extraction would have resulted in larger recovery, but it was felt that this was a reasonable practical limit.

A comparison of the amount of residual extractives in each case shows clearly the effect of higher extraction temperature. Equilibrium was invariably reached more quickly during reflux due to this higher temperature; and the fact that the return of condensate to the system had the effect of introducing small quantities of fresh solvent as the extraction progressed.

It should be pointed out that the extraction periods in this preparation were excessively long. Three or four extraction periods of 2-3 hours would very likely be adequate to reach the same extraction limit.

STEAMING OF THE WOOD

When the final extract had been drawn off from each of the wood charges, steam was admitted directly at the base of the extractor. Within a few minutes (Table III), alcohol vapors passed to the condenser, and condensate was collected in the 125 gallon still.

The first wood charge was steamed quite slowly, and the nearly constant rate of removal of high strength alcohol is shown in Table III. As would be expected, larger steam demands were noted for the initial recovery; and the steam requirements decreased as the alcohol was boiled off and a steady rate was reached.

TABLE III

STEAMING OF SPENT WOOD CHARGE NO. 1

Time (min.)	Meter (lbs.)	Demand (no./gal.)	Alcohol Volume (gal.)	Cumulative Rate (g.p.m.)	Per Cent Alcohol
—	360	—	0	—	—
10	—	—	1	.10	95
20	390	10	3	.15	95
55	429	5.2	9 1/2	.17	95
175	629	5.0	30	.17	0

Data taken during the steaming of No. 2 charge were somewhat erratic, and are thus not included in this resumé. During the No. 2 steaming, however, the effects of attempts to speed the recovery were noted. As the steam flow was increased, the alcohol strength fell off sharply, due, apparently, to the accompanying decrease in the effectiveness of the wood charge as a packed section. The steaming time was thus reduced by only a few minutes, while the time required to rectify the recovered solvent was increased by 25%-30%.

From the standpoint of solvent recovery, this direct steaming is quite beneficial in that essentially complete recovery is realized. It would also be economically practical.

CONCENTRATION OF THE EXTRACT

When the extract had reached equilibrium concentration, and had been pumped through the filter press, it was collected in the copper still to be concentrated before further handling.

In concentrating the first extract, alcohol was recovered until a solution that was 10% solids by weight was left. There was leakage, and accompanying air-binding of the reflux pumps when the system was operated at reduced pressure; but precipitation and clogging troubles in the still were not immediately apparent.

It was at first expected that the remaining concentrate

could be treated with as little trouble. The higher concentration of the extract, however, was not given adequate consideration, and there were heavy deposits of alcohol-soluble concentrate in the still before recovery was stopped. When the amorphous extract had been removed, concentration was continued until the remaining liquor was essentially a water extract.

Throughout the distillation operation, considerable difficulty was experienced in maintaining the recovery rate. Leaks in pump packing and throughout the reflux system caused pumps to become air-bound from time to time and resulted in there being unmeasured losses into the vacuum line when correction could not be made immediately. Final distillation after pumps were repacked was much improved, however, and it is felt that close observation of distillation operation in the future can prevent solvent losses as were experienced in this preparation.

The final extract as submitted for study consisted of 3 samples as follows:

(1) 150 gms.	dry
(2) 5902 gms.	73% Solids
(3) 3150 gms.	20% Solids

or a total of 5101 gms. (about 11.3 pounds) of combined extractives. In estimating a total yield of concentrate, approximately 2 pounds should be included which was lost in an accidental spillage of water

extract which occurred late in the process.

A total of 13.3 pounds of extractives, or 82.7% of the total alcohol extractives were considered to have been removed.

Overall solvent recovery was as follows:

Initial Solvent		Recovered Solvent		
Per Cent Alcohol	Volume (gal.)	Volume (gal.)	Per Cent Alcohol	95% Equivalent
95	162	42.0	92	40.8 gal.
		45.5	90	43.1
		42.4	85	38.0
		9.0	32	3.1

Per Cent Recovery 77.0%

Since a maximum of 0.5 gallons of alcohol can be accounted for in the 73% extract, it must be assumed that the relatively low solvent recovery was due to mechanical losses. There was leakage due to loose pipe connections which were remedied as quickly as possible; and some spillage in transferring small volumes of solvent or extract. The greatest mechanical losses, however, are believed to have occurred when the reflux pump became air-bound. When reflux was not returned to the tower, the vent-bottle at the base of the 8" condenser readily became full whereupon alcohol could be sucked into the vacuum line.

As no economic study was intended in conjunction with this preparation, no additional utility measurement equipment was installed. Observations were made, however, of overall steam and water demands. It is felt that the general results of these observations may be of interest although not necessarily applicable in considering comparable extractions in the future.

In conducting the extractions, 3300 pounds of steam were used; and water consumption amounted to 66,400 gallons. On a "per pound of extractives" basis, the above may be expressed as 292 pounds of steam and 6000 U. S. gallons of water per pound of recovered dry extract — or 250 pounds of steam and 5100 gallons of water per pound of assumed total extract.

In general, it would seem that with some preliminary study, alcohol extracts of Western Red Cedar could be produced quite efficiently through application of a suitable continuous countercurrent extraction battery.

dhh/mak

NOVEMBER 1945

PROJECT REPORT FORM

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COOPERATOR Institute
REPORT NO. 3
DATE October 30, 1945
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ATTEMPTS TO ISOLATE THE COLOR PRINCIPLE IN SOUTHERN RED OAK WOOD

Because of the strong color test of southern red oak wood with methanol-hydrochloric acid solution, this species was chosen as a representative of the hardwoods to be investigated.

Sawdust was prepared from a southern red oak log received from the Crossett Lumber Company in 1944. Both sapwood and heartwood were used. Sawdust was used in experiments I, III, IV, and V, but a portion ground in a Wiley mill to pass 40 mesh was used in experiment II. The moisture content of the sawdust was 11.5% and of the woodmeal 4.4%

Southern Red Oak Extraction I.

The sawdust (approximately 6.0 g. on the oven-dry basis) was extracted with ether in a Soxhlet for 10 hours. The ether extract was yellow; when evaporated to dryness the residue did not give a positive color test with methanol-HCl.

The ether extracted wood was next extracted with acetone for 16 hours (2 days). After removal of the acetone the residue was treated with ether. Both the ether soluble and ether insoluble fractions gave a strong purple color immediately with methanol-HCl. Since the sawdust gave a test with methanol-HCl it was extracted for 85 hours (11 days; total, 101 hours) with fresh acetone. The acetone extract was evaporated almost to dryness and treated with ether. The ether solubles gave a

deep red color and the ether insolubles gave an orange-brown color in the methanol-HCl test. The sawdust gave a test with methanol-HCl in a few minutes. Again using fresh acetone the extraction was continued until the total time with acetone was 128 hours. The extractive obtained on evaporation of the solvent was treated with ether. Both the ether soluble and ether insoluble fractions gave a yellow-brown color with methanol-HCl. The sawdust still gave a decided red test.

The ether and acetone extracted wood was extracted with methanol for 16 hours (2 days). The deep yellow extract was evaporated to dryness and the residue treated with ether; it was practically insoluble. The ether soluble fraction did not give a test with methanol-HCl but the ether insoluble fraction gave an intense red color in a short time. The sawdust slowly gave a faint pink color test. It was extracted with fresh methanol for 17 more hours (2 days) but still gave a faint pink test. Again the extractive was almost completely insoluble in ether and gave an intense red color with methanol-HCl.

Fresh methanol was added and the extraction continued for 39 hours (5 days; total, 72 hours). The concentrated extract gave a pale purple test with methanol-HCl in a short time. Since the sawdust still gave a faint test the extraction was continued for 48 hours (6 days; total 120 hours) with fresh methanol. The faint yellow extract when evaporated to dryness gave an orange color with a slight red tint when treated with methanol-HCl. The sawdust gave a very faint test.

Fresh methanol was used for 89 additional hours extraction (11 days; total 209 hours). The extractive gave a questionable test with methanol-HCl and the sawdust gave a very faint test. The last methanol extraction was made for 91 hours (11 days; total 300 hours) with fresh methanol. The pale yellow extract was evaporated to dryness and the residue tested with methanol-HCl solution. An orange color resulted and did not change when a spruce splinter was added. The sawdust still gave a very faint test with methanol-HCl.

The ether, acetone, and methanol extracted sawdust was extracted three times for 8-hour periods with distilled water. The extractive did not give a test with methanol-HCl but the sawdust still gave a very faint pink test.

This experiment indicates that the constituent responsible for the color test with methanol-HCl is insoluble in ether (at least in the original wood) but soluble in acetone and methanol. The solubility appears to be higher in methanol. It is also evident that prolonged extraction with these solvents will not remove the component entirely from the sawdust.

Southern Red Oak Extraction II.

The wood meal (95.6 g. on oven-dry basis) was extracted with 1 liter of water at 93° for 6 hours and then separated by suction. The extract was yellow brown; it will be designated as II a. The extracted wood meal was again treated with water at 93° for 6 hours and filtered

by suction. The extract is known as II b. Both extracts were subjected to a scheme of separation and tests with methanol-HCl made at various strategic points. Starch was found by the iodine color test in these water extracts.

Extract II a was evaported to dryness. The orange brown residue gave an intense red color with methanol-HCl. This residue was redissolved in the minimum quantity of water (it was questionable whether the small amount which remained insoluble gave a test with methanol-HCl). The water solution was extracted with ether. The ether soluble fraction was pale yellow. It gave an ephemeral pale pink color test. This ether solution was then extracted with 1% ammonium hydroxide. The ether soluble fraction was colorless and gave no color test. The dilute ammonia fraction was a deep yellow color with a greenish cast. On acidification to pH 4 it became yellow. This acidified solution was again extracted with ether. The resulting ether solution was faint yellow and gave no color test; the extraction with dilute ammonia was repeated on this fraction. The acid fraction was orange colored and did not give a color test.

The water soluble fraction (insoluble in ether) was orange colored. It was heated to remove the dissolved ether. The color test with methanol-HCl was questionable. After partial concentration it was poured into five volumes of methanol. The light brown precipitate which formed did not give a color test. The methanol fraction was orange colored. It gave a questionable color test which when repeated gave a

definite red tinge. On evaporation to dryness a brown residue was obtained (Sample 61939 M). This residue did not give a color test.

Extract II b was not evaporated to dryness but was extracted directly with ether. The ether soluble fraction was faint yellow. It gave a pale pink test with methanol-HCl which disappeared in 20 minutes. The ether solution was evaporated almost to dryness until only the dissolved water remained. This solution was orange in color and contained a white precipitate. It was extracted twice with ether. Since the orange color and white precipitate were insoluble in ether they probably had been carried in dissolved water solution. The orange colored water solution slowly gave a pink test with methanol-HCl but the white solid did not. The ether soluble fraction was colorless. It gave a color test. It was further separated by extraction with 1% ammonium hydroxide. The fraction remaining in ether solution was faint yellow; it gave an ephemeral color test. The portion extracted with ammonia was bright yellow in color and when the solution was acidified it turned faint yellow. It was again extracted with ether. Neither fraction gave a color test with methanol-HCl.

The water soluble fraction (ether insoluble) from Extract II b was orange brown. The ether was removed by heating and concentrated to one-half volume. An aliquot evaporated to dryness gave a color test with methanol-HCl. It was the methanol soluble part which carried the component. Another aliquot was hydrolyzed briefly with HCl and then

extracted with isoamyl alcohol. The acid layer was a light brown color and the alcohol layer was a reddish purple color. When ether was added to the solution it drove the color into the water layer which then appeared orange brown. A third aliquot was treated directly with isoamyl alcohol. The pale yellow alcohol layer gave a pale red color with methanol-HCl and the orange brown water layer gave a questionable color test. The remainder of the solution was evaporated to dryness. The dark brown residue (Sample 61939 W) gave a faint color with methanol-HCl.

The water extracted wood meal was air-dried and extracted in a Soxhlet with methanol for a total of 100 hours (12 days). The methanol was changed after 30 hours and 60 hours. The extract gave a strong color test after 30 hours, a medium test after 60 hours and a pale red test after 100 hours. The extracted wood meal gave a color test in a couple minutes after the 30 hour period and a pale violet in 5 minutes after the 60 hour and 100 hour period. The first two fractions (up to 60 hours) were combined for study but the third fraction was handled alone.

The combined fraction was evaporated to dryness (1.1 g.; 1.15% on wood) and mixed thoroughly with ether several times. About 30% (0.326 g.) was soluble in ether and gave a yellow solution. It did not give a color test with methanol-HCl (Sample 61931 E). The portion insoluble in ether (0.776 g.) was red brown in color. It was almost completely redissolved in methanol and gave a color test. (Sample

61931 M). A small amount of this was treated with isoamyl alcohol and water. The alcohol fraction gave a questionable color test, the water fraction gave a negative test and an insoluble fraction gave a distinct color test with methanol-HCl. Another aliquot of this ether insoluble material was extracted with water for 72 hours, filtered, the insoluble part was hydrolyzed with dilute HCl on the steambath for 4 hours, cooled and extracted with ether. The ether solubles did not give a color test, the water solubles (in acid) gave a faint test and the unhydrolyzed portion gave a faint test. The hydrolysis was repeated on another aliquot with 1% H_2SO_4; this time the water solubles did not give a color test.

The other methanol extract was concentrated in vacuo and the methanol replaced with approximately 100 cc. of water. Half of this solution was extracted with an equal volume of isoamyl alcohol. Neither the alcohol nor the water fractions gave a color test and it was questionable whether the insoluble fraction gave a faint test with methanol-HCl. The other half was filtered and evaporated to dryness. The light brown residue (Sample 61939 MB) did not give a color test.

The attempt to separate the water extracts into various fractions did not lead to definite results probably because the separations were not sharp enough. A flavone was apparently present, but this did not appear to give the color test with methanol-HCl. Indications were that a combination of isoamyl alcohol and water could be used to effect partial elimination of extractives obtained with methanol which did not give a color test.

Southern Red Oak Extraction III.

The sawdust (381 g. on oven-dry basis) was extracted with methanol in a Soxhlet for 16 hours (2 days). The orange brown extract (vol. 750 cc.) contained 16.38 g. of solids of which 0.68 g. was a yellow precipitate which settled out on cooling. The yellow precipitate did not give a color test with methanol-HCl; it was saved for further study.

The methanol of the solution was replaced by water which formed a suspension. This was extracted with isoamyl alcohol several times until the alcohol layer was practically colorless. The isoamyl alcohol was removed by evaporation in vacuo. When the volume had been reduced to 200 cc. a light colored precipitate settled out. This was removed (0.489 g.; Sample 61949 A) and the evaporation continued to dryness. A dark red-brown solid resulted (6.522 g.; Sample 61949 B). The light colored precipitate gave a strong test with methanol-HCl but the dark colored evaporate did not. Unfortunately the isoamyl alcohol extract had not been filtered before concentration so it was not clear if the extract contained two fractions or if the light colored material had been in fine suspension.

The aqueous layer and insolubles were extracted with ether twice primarily to remove the dissolved isoamyl alcohol. On evaporation of the ether layer a slight residue was obtained which did not give a color test.

The aqueous layer was separated from the fraction insoluble

in isoamyl alcohol, ether, and water. When evaporated to dryness a brown solid was obtained (8.07 g.; Sample 61949 W) which did not give a color test.

The insoluble fraction was redissolved in methanol. It could not be dried in vacuo at 60° or by long standing in a vacuum desiccator. This brown syrup (Sample 61949 I) did not give a color test.

The extracted sawdust was extracted again with fresh methanol for 46 hours (total 62 hours). The extract had a solids content of 6.04 g. The methanol was replaced with water and the suspension extracted with isoamyl alcohol and ether. No precipitate separated from the alcohol layer during concentration and the dark red brown evaporate (3.083 g.; Sample 61951 A) did not give a color test. The ether extractive was negligible and gave no test. The water solubles were brown on evaporation (2.37 g.; Sample 61951 W) and did not give a color test. The fraction insoluble in isoamyl alcohol, ether and water was tan colored (0.586 g.; Sample 61951 M) and gave a color test with methanol-HCl. The foregoing data are summarized in Table I with those of extractions IV and V.

Southern Red Oak Extraction IV.

The sawdust (413 g. on oven-dry basis) was extracted with methanol in a Soxhlet for 17 hours (2 days). The orange brown extract (vol. 760 cc.) contained 20.4 g. of solids of which 0.245 was a yellow precipitate which settled out on cooling. The yellow precipitate did

not give a color test with methanol-HCl; it was saved for further study.

The methanol of the solution was replaced by water which formed a suspension. This was extracted with isoamyl alcohol several times until the alcohol layer was practically colorless. The aqueous layer and insolubles were extracted with ether twice and the negligible solids added to the isoamyl alcohol layer. The alcohol was filtered before concentration. On concentration to low volume a precipitate settled out and was removed (8.03 g.; Sample 61956 A). Evaporation was continued to dryness (1.85 g.; Sample 61956 B). Both of these samples were red brown in color and gave only a faint test with methanol-HCl.

The aqueous layer was separated from the fraction insoluble in isoamyl alcohol, ether and water. When evaporated to dryness a dark brown solid was obtained (8.00 g.; Sample 61956 W) which did not give a color test.

The insoluble fraction was obtained in two parts, the solids filtered out of the alcohol and water layers, and the solids removed from the separatory funnel with methanol. The former was salmon pink (0.776 g.; Sample 61956 I-1) and gave a strong color test with methanol-HCl. The latter was a brown solid with a reddish tinge (0.494 g.; Sample 61956 I-2) and gave a weak color test.

The extraction of the sawdust was continued with fresh methanol for 51 hours (total 68 hours). The extract (vol. 1650 cc.) contained 2.86 g. of solids. The methanol was replaced with water and the suspension extracted with isoamyl alcohol and ether. These layers were combined and filtered to remove suspended insolubles. The evaporate from the alcohol fraction was a red brown solid (1.678 g.; Sample 61960 A) which gave a faint color test with methanol-HCl. The water layer was also filtered and evaporated to dryness; a brown solid resulted (0.567 g.; Sample 61960 W) which did not give a color test. The fraction insoluble in isoamyl alcohol, ether and water was colored light brown (0.315 g.; Sample 61961) and gave a color test with methanol-HCl. The above data are summarized in Table I.

Southern Red Oak Extraction V.

The sawdust (382 g. on oven-dry basis) was extracted with methanol in a Soxhlet for 21 hours (3 days). The orange brown extract (vol. 1770 cc.) contained 19.47 g. of solids. It was concentrated in vacuo to a volume of 500 cc. and the yellow precipitate which settled out removed by filtration. The yellow precipitate (0.757 g.) did not give a color test with methanol-HCl; it was saved for further study.

The methanol was replaced by water and the suspension extracted with isoamyl alcohol until the alcohol layer was nearly colorless. The alcohol layer was filtered to remove insolubles in fine suspension and then evaporated to dryness. The evaporate was red brown (8.68 g.;

Sample 61967 A) and gave a faint color test.

The aqueous layer was filtered from the insolubles. When evaporated to dryness a brown solid was obtained (8.86 g.; Sample 61949 W) which did not give a color test.

The insolubles, including the filtered portions and the part dissolved in methanol, were taken to dryness. The brown solid (the filtered parts had been salmon pink before combination) amounted to 1.166 g. (Sample 61967 I). It gave a color test with methanol-HCl. The above data are summarized in Table I.

ihi/sgt

TABLE I

Yields of Fractions in Methanol Extractions of Southern Red Oak

	Experiment III			Experiment IV			Experiment V
	1	2	Total	1	2	Total	
Wood, g.	381		381	413		413	382
Extraction time, hr.	16	46	62	17	51	68	21
Total solids, g.	16.38	6.04	22.42	20.40	2.86	23.26	19.47
Total solids, % on wood	4.30	1.59	5.89	4.95	0.69	5.63	5.10
Yellow precipitate, g.	0.68	0	0.68	0.245	0	0.245	0.757
Yellow precipitate, % on wood	0.18	-	0.18	0.06	-	0.06	0.20
Yellow precipitate, % of solids	4.2	-	3.0	1.2	-	1.1	3.8
Yellow precipitate, color test	No			No			No
Isoamyl alcohol soluble, g.	6.82	3.08	9.90	9.88	1.68	11.56	8.68
Isoamyl alcohol soluble, % on wood	1.79	0.81	2.60	2.39	0.41	2.80	2.27
Isoamyl alcohol soluble, % on solids	41.6	51.0	44.2	48.4	58.8	49.7	44.6
Isoamyl alcohol soluble, color test	No	No		Faint	Faint		Faint
Water soluble, g.	8.07	2.37	10.44	8.00	0.87	8.87	8.86
Water soluble, % on wood	2.11	0.62	2.73	1.94	0.21	2.15	2.32
Water soluble, % on solids	49.3	39.2	46.6	39.2	30.4	38.2	45.6
Water soluble, color test	No	No		No	No		No
Insolubles, g.	0.489[1] 0.321[2]	0.586	1.40	0.776 0.494	0.315	1.585	1.166
Insolubles, % on wood	0.13 0.08	0.15	0.37	0.19 0.12	0.08	0.39	0.30
Insolubles, color test	Strong No	Yes		Strong Weak	Yes		Yes
Insolubles, % on solids	4.9	9.8	6.2	6.2	10.8	6.8	6.0

1) Isoamyl alcohol fraction precipitates which had not been removed by filtration.
2) A syrup; this weight obtained by difference.

PROJECT REPORT FORM

PROJECT NO. 1127
COOPERATOR Institute
REPORT NO. 2
DATE October 1, 1945
NOTE BOOK 619
PAGE 27 TO 65
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ATTEMPTS TO ISOLATE THE MATERIAL RESPONSIBLE FOR THE COLOR TEST

The previous work had indicated that the color test which certain woods give with methanol-hydrochloric acid might be due to the presence of some simple material like phloroglucinol or indole which reacts with the lignin in the presence of hydrochloric acid. Such a material would probably be ether soluble. Attempts to isolate such an ether soluble material from Western hemlock were unsuccessful. However the material responsible for the color formation was concentrated in a fraction insoluble in isoamyl alcohol, ether, and water. This fraction contains slightly more than 11% methoxyl and may be contaminated with native lignin. A similar fraction was obtained from black spruce but the fraction from spruce does not give the color test.

Sawdust was prepared from a Western hemlock log received from Crown Zellerbach Corporation in 1943 or 1944. Both sapwood and heartwood were used. The sawdust was ground in the Wiley mill. The resulting wood meal contained 13.5% moisture.

Extraction of Western hemlock I:

The wood meal (368 g. on the oven-dry basis) was extracted with ether in a Soxhlet for 24 hours (3 days). On concentrating the ether extract to about 30 ml. colorless platelike crystals separated. The yield was 0.15 g. or 0.04%. These crystals melted at 60-65° C., and after one

recrystallization from acetone melted at the same temperature. They were soluble in ether, but insoluble in cold acetone or alcohol. These crystals did not give a color with methanol-Hcl or a spruce splinter.

The remainder of the ether extract on drying weighed 3.3 g. (total ether soluble 0.95%). This residue was mixed with 35 ml. of methanol and the methanol solution separated from 0.3 g. of methanol insoluble material similar to the crystalline material described above. The methanol soluble material did not give a color with methanol-Hcl or with a spruce splinter.

The ether extracted wood was next extracted with methanol for 24 hours (3 days). On removing the methanol 13.5 g. of material were obtained. This was separated into ether soluble material (5.7 g.; 1.5%) and ether insoluble material. The ether soluble materials did not give a test with methanol-Hcl or with a spruce splinter. The ether insoluble material gave a test with methanol-Hcl.

The methanol extracted wood was extracted with fresh methanol for 50 hrs. (9 days). The wood still gave a test with methanol-Hcl, both the wood and the solution being colored. The second methanol extract was concentrated, 500 ml. of water added and concentrated again to remove the methanol. The resulting cloudy aqueous system was extracted with isoamyl alochol, which removed 0.6 g. of material which was soluble in methanol to give a red colored solution. The addition of methanol-Hcl appeared to intensify this color.

The aqueous system was next extracted with ether which removed 0.1 g. of material which did not give a test with methanol-Hcl. After the ether extraction a small amount of red colored insoluble material was in the separatory funnel. This gave a strong test with methanol-Hcl.

The extracted aqueous layer was treated with one-tenth volume of 10% sulfuric acid. Addition of the acid caused the formation of a floc which was probably due to coagulation of a colloidal material. The mixture was hydrolyzed by boiling for 2 hours. Ether extracted 0.1 g. of material from the hydrolyzed solution but this did not give a test with methanol-Hcl. The hydrolyzed aqueous layer was next extracted with isoamyl alcohol. This removed 0.1 g. of dark material which was partly soluble in methanol to give a deep red color. This color may have been due to the presence of sulfuric acid in the residue.

After the amyl alcohol extraction, a red precipitate was present in the aqueous layer. This was mostly insoluble in methanol. The methanol soluble part formed a red brown solution, but the addition of methanol-Hcl did not appear to intensify the color.

This preliminary experiment was made to determine whether or not Western hemlock contains some material such as phloroglucinol which reacts with lignin in the presence of hydrochloric acid to give the characteristic color reaction. Such a material should be ether

soluble, but in view of Ertmann's work on pinosylvin it might not be removed directly from the wood but would be removed by an alcohol extraction. Instead of existing in the free form it might exist as a glucoside and hence would be ether insoluble, until after hydrolysis. No evidence was found for the presence of such a material, but extraction with amyl alcohol as carried out in this experiment appears to offer a means of separating the material responsible for the color formation from the bulk of the native lignin.

Extraction of Western Hemlock II:

In order to isolate more of the isoamyl alcohol, ether, and water insoluble fraction 470 g. (412 g. on the o. d. basis) of Western hemlock were extracted with methanol for 16 hours (2 days). The first methanol extract was separated and filtered. The wood was next extracted with fresh methanol for an additional 44 hours (1 week). The two extracts were worked up separately as described below.

An aliquot of the methanol solution was evaporated to dryness in order to determine the amount of solids extracted. The remainder of the solution was concentrated, 1000 ml. of water added and the remainder of the methanol removed under reduced pressure. The aqueous layer with the precipitated solids was extracted 4 times with isoamyl alcohol. The combined isoamyl alcohol extracts were concentrated to dryness under reduced pressure. The yields of the various fractions are given in Table I.

TABLE I

YIELD IN GRAMS OF FRACTIONS FROM SECOND EXPERIMENT

	First Extract	Second Extract	Total*
Total extracted solids	16.1	2.3	18.4 (4.5)
Isoamyl alcohol soluble fraction	13.4	1.9	15.3 (3.7)
Ether soluble fraction	0.8	0.1	0.9 (0.2)
Water soluble fraction	1.5	0.2	1.7 (0.4)
"Insoluble" fraction	0.4	0.09	0.5 (0.1)

* The values in parenthesis are expressed as per cent of the original wood oven-dry basis.

The aqueous layer was next extracted five times with ether. The combined ether extracts were combined and concentrated to dryness. The aqueous layer was then filtered and the filtrate concentrated to dryness under reduced pressure.

During the extractions a reddish colored insoluble material had separated. This was dissolved in a mixture of dioxane and methanol. The methanol was removed by concentrating under reduced pressure. The resulting dioxane solution was centrifuged, filtered, and dropped into anhydrous ether. The product which separated was washed with ether and then with petroleum ether.

One per cent solutions of the various fractions in methanol were tested with methanol-2% hydrochloric acid, except the ether soluble fraction was not completely soluble in methanol and 1:1 methanol-acetone was used as the solvent in this case. The ether soluble and water soluble fractions both failed to give a test with methanol-hydro-

chloric acid, and a spruce splinter was not colored. The isoamyl alcohol soluble fraction gave a slight test and the insoluble fraction gave a strong test with methanol-hydrochloric acid. Thus the substance responsible for the color formation appears to be concentrated in the insoluble fraction, but a part of the material is in the isoamyl alcohol soluble fraction.

The isoamyl alcohol soluble fraction probably contains the bulk of the native lignin. Whether or not the insoluble fraction contains native lignin or not is unknown. The methoxyl contents of the insoluble fractions from the first and second extractions are 12.6 and 11.3% respectively. These values indicate that the insoluble fractions contain about 80% native lignin or some other methoxyl containing material. The material responsible for the color formation may contain methoxyl groups.

Extraction of Western Hemlock III:

A third portion of Western hemlock was extracted in order to obtain a sample of the insoluble fraction for purification. Because of the difficulty in separating the native lignin from the substance responsible for the color formation, the wood was heated in an oven at 105° C. for 45 hours in the belief that this would decrease the amount of lignin extracted. The ovendry wood (400 g.) was extracted with methanol for 8 days, the extract being replaced with fresh methanol after the second day.

Both methanol extracts were filtered hot in order to separate any wood meal. On cooling a small amount of methanol insoluble material separated. This material was soluble in ether, insoluble in water, cold methanol, or acetone, and appeared to be the same as the crystalline material obtained from the ether extract in the first experiment.

The two methanol extracts were combined, concentrated, 1000 ml. water added, and the remainder of the methanol removed under reduced pressure. The resulting aqueous system was extracted 5 times with isoamyl alcohol. On concentrating the amyl alcohol to about 200 ml. a small amount of material had precipitated. This was separated and the amyl alcohol filtrate concentrated to dryness under reduced pressure.

The aqueous layer was next extracted with ether and was finally filtered, but the ether soluble and the water soluble fractions were not isolated. The yields of the fractions which were isolated are given in Table II.

TABLE II

YIELD OF FRACTIONS FROM THIRD EXPERIMENT

	Wt. g.	% of o. d. Wood
Methanol insoluble fraction	0.1	
Total methanol soluble solids	17.9	4.5
Isoamyl alcohol soluble fraction	16.2	4.1
Fraction which separated on concentrating amyl alcohol	0.4	0.1
"Insoluble" fraction	0.3	0.1

Both the insoluble fraction and the fraction which separated on concentrating the amyl alcohol were dissolved in 100 ml. methanol. The latter fraction was readily soluble in methanol, but a small amount (2 ml.) of dioxane was added in the first case in order to dissolve the material. Samples were isolated for methoxyl determinations by taking aliquots of the solutions, replacing the methenol by dioxane, and precipitating into ether. The remainder of each fraction was purified by adding water to the methanol, removing the methanol under reduced pressure, and extracting the resulting aqueous system with isoamyl alcohol and ether according to the general procedures used in isolating the original fractions. The methoxyl contents of the fractions and the yields of the purified fractions are given in Table III.

Tests with methanol-hydrochloric acid were made by dissolving 1 mg. of the various fractions in 0.2 ml. methanol and 1 drop of dioxane. All of the fractions were completely soluble. This corresponds to concentration of 0.4 - 0.5%. An equal volume of methanol-2% hydrochloric acid was added to each. The results are indicated in Table III.

TABLE III

PURIFICATION OF FRACTIONS

	Test with Methanol-Hcl	MeO%	Yields % of Original Fraction
Fraction which precipitated on concentrating amyl alcohol	Faint	13.7	
Purification of above:			
Amyl alcohol soluble fraction			68
Fraction precipitated on conc.	Very faint	14.7	12
Insoluble fraction	Medium	12.5	6
"Insoluble" fraction	Medium	11.1	
Purification of above:			
Amyl alcohol soluble fraction			33
Insoluble fraction	Strong	11.1	54

The data in Table III indicate that the fraction which precipitated on concentrating consists largely of material soluble in amyl alcohol, and that a small amount of the so-called insoluble fraction is present. This "insoluble" part has slightly less methoxyl than the total fraction and contains a higher concentration of tne material responsible for the color formation.

The methoxyl content of the "insoluble fraction" is about the same as that (11.3%) of the corresponding fraction isolated from the second extrection in the second experiment. Purification of this fraction failed to change the methoxyl but appeared to increase the concentration of the material responsible for the color formation. Because the methoxyl

did not change it is impossible to say whether this fraction consists chiefly of material having a high methoxyl which is responsible for the color fraction or whether the fraction consists of about 80% native lignin mixed with a small amount of the color producing substances.

Extraction of Black Spruce:

Black spruce wood meal was dried in an oven for 45 hours, and the dried wood meal (290 g.) was extracted with methanol in a Soxhlet. After 2 days the extract was replaced with fresh methanol and the extraction continued for one week (total extraction time 60 hrs.). The second extract contained only 0.6 g. solids. The extracts were combined, the methanol replaced with water, extracted with isoamyl alcohol and ether in the manner described for the first extract in the second experiment on Western hemlock. The yields of the various fractions are given in Table IV.

TABLE IV

YIELDS OF FRACTIONS FROM BLACK SPRUCE

	Wt. g.	% of o. d. Wood
Total extracted solids	7.4	2.5
Isoamyl alcohol soluble fraction	4.4	1.5
Ether soluble fraction	0.1	0.03
"Insoluble" fraction	0.04	0.01
Water soluble fraction	2.9	1.0

As indicated in Table IV a small amount of the so-called insoluble fraction was obtained from black spruce, but the yield was only about one-tenth of the yield of the corresponding fraction from Western hemlock. The insoluble fraction from spruce was buff rather than reddish colored and did not give a test with methanol-hydrochloric acid. The methoxyl content of this fraction was 16.1%.

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COOPERATOR Institute
REPORT NO. 1
DATE October 1, 1945
NOTE BOOK 619
PAGE 9, 26, 30 TO
SIGNED M. A. Buchanan

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FACTORS INFLUENCING THE COLOR TEST

The effect of using different solvents and acids on the color produced with certain woods was reported in the Research Bulleton, vol. 11, no. 3, p. 163. This report describes a few additional factors.

Increasing the pH to 4-4.5 destroys the typical color. The addition of small amounts of formaldehyde to ethanol does not make ethanol equivalent to methanol. Small amounts of phloroglucinol, indole, or skatole develop strong colors on black spruce wood in the presence of methanol-hydrochloric acid. The addition of concentrated hydrochloric acid alone to black cherry heartwood produces a deep red color indicating the presence of phloroglucinol or some similar substance in this wood.

Effect of neutralization on the color test: Basswood sawdust was allowed to stand with methanol-hydrochloric acid for 24 hours. On the addition of sodium acetate the typical violet color changed to a yellow at a pH of about 4-4.5. On standing the yellow color disappeared leaving a nearly colorless mixture. A basswood shaving, after soaking in methanol-hydrochloric acid for 24 hours, was exposed to the vapors from 6 N ammonium hydroxide. The typical violet color changed to a yellow and then to the color of the original wood.

Effect of formaldehyde in ethanol: Methanol appears to be unique as a solvent for the color tests. It has been suggested that

this may be due to small amounts of formaldehyde in the methanol used. The addition of 1 ml. and 0.1 ml. of formalin to 20 ml. portions of absolute ethanol gave the same result with Western hemlock as was obtained with ethanol.

Substances which give a test with spruce: Many materials are known which give a color test with spruce lignin in the presence of aqueous hydrochloric acid. A few of these materials were tested in the presence of methanol-hydrochloric acid. Black spruce sawdust in a test tube was covered with methanol-1% hydrochloric acid and a small amount of the material to be tested was added with the following results:

1. Resorcinol - slight violet formed quickly; lavender in two hours.

2. dl-Tryptophane - similar to blank.

3. Phloroglucinol - immediate deep red; red-purple after 24 hours, yellow solution.

4. Indole - immediate blue red; red after 2 hours, orange solution.

5. Skatole - red formed quickly; changed to a red brown; blue solution.

6. Quercitrin - yellow solution.

7. Digitonin - similar to blank.

8. Blank - a light pink developed slowly, probably due to presence of some inner bark.

As indicated above phloroglucinol, indole, and skatole give strong tests with spruce in the presence of methanol-hydrochloric acid. None of these colors were identical to that produced in the typical color test and in each case the color of the solution differed from that of the wood.

In order to determine the concentration necessary to produce a color various phloroglucinol and indole solutions in methanol-hydrochloric acid were added to black spruce splinters. The results are given in Table I.

TABLE I

Effect of Concentration of Phloroglucinol and Indole on Color Formation

	Concentration, %	Time to Form Color	Color after 20 hours
Phloroglucinol:			
	0.1	10 sec.	deep red
	0.01	20 sec.	deep red
	0.001	2-3 min.	red-violet
	0.0001	>2 hours	lavender-grey
Indole:			
	0.1	10-15 sec.	red-brown
	0.01	20-30 sec.	red-brown
	0.001	2-5 min.	deep red
	0.0001	>1.5 hours	deep-red blue

The above data indicate that a concentration of phloroglucinol or indole in the solution in the order of 0.001% is sufficient to produce a strong color with spruce wood. Indole appears to be effective in slightly lower concentration than is phloroglucinol.

Tests with concentrated aqueous hydrochloric acid: Concentrated hydrochloric acid added to shavings of black cherry heartwood quickly developed a red color. A red color did not develop with red maple or birch under the same conditions.

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